Survey: puppy feeding practices and perceptions in Switzerland

Opsomer, Han ; Gerstner, Kerstin ; Liesegang, Annette

Abstract: extended abstract

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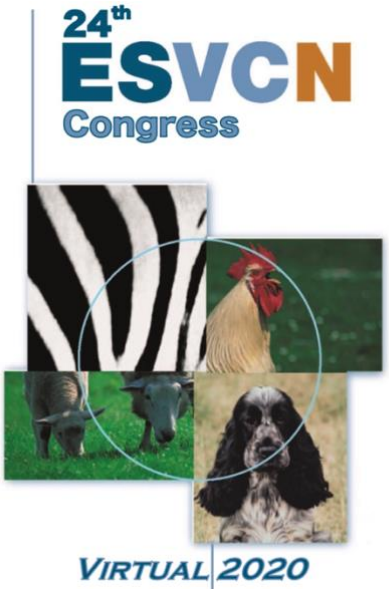

Congress proceedings

24th Congress of the European College of Veterinary and Comparative Nutrition

September 17 – 19, 2020

Online, hosted by Ludwig-Maximilians-Universität München

Hosted by: Ludwig-Maximilians-Universität München

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Welcome

Dear Colleagues,

This year was and still is a very challenging year - confronting us with a new reality, nobody could have imagined possible. Covid-19 changed (professional and private) lives globally. First of all, we hope that you and your loved ones are all safe and unharmed and our heart goes out to all affected by this threat.

As it became more and more obvious in the spring of 2020 that this year's congress would not be possible in the normal format at the planned venue in Portugal, the ESVCN Board had to decide how to proceed. Weighing pros and cons and discussing all implications, finally the decision was made to go for a virtual congress instead of having no congress at all this year. As there is a positive side to virtually all situations, we are sure that this new format also has its advantages. A virtual congress not only provides the space and the stage for our scientific exchange, education and broadening of knowledge, it might also attract nutritionists who had not yet been able to attend one of our congresses. Traveling to a congress allows direct contact, conversation and, with this, renewing or deepening of existing contacts. However, it also costs time, money and resources. Covid19 will change the world permanently. I hope that we will keep only the smart things, we learned the hard way, and get rid of the restrictions, threads and annoyances so many of us had and still have to endure.

The 24th Congress of the European Society of Veterinary and Comparative Nutrition is hosted by the Ludwig-Maximilians-Universität Munich – not in the historical buildings as in 2018 but using the university server and IT infrastructure. Out of this year´s 100 submissions in the broad field of veterinary and comparative nutrition, which were evaluated in a thorough reviewing system, as always, 39 contributions will be presented orally and 61 as posters. The Society is grateful for the ongoing support from our sponsors and supporters, enabling us to successfully organise such a growing international event of high scientific value and increasing visibility.

On behalf of the ESVCN Board, I want to express my hope that the 24th congress will be equally stimulating, informative and enjoyable.

Britta Dobenecker

President, ESVCN

Local organizing committee

Chair: Britta Dobenecker

Virtual platform: Andrea Ellis, Linda Böswald, Georgina Crossman

Website: Andrea Ellis

Programme: Ronald Jan Corbee

ESVCN Board

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Award Winners ESVCN 2019

Helmut Meyer Award - In remembrance of the late Prof. Helmut Meyer

Best oral presentation on a general topic.

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Marco Fratinati *et al*.: Dietary regimen of the wood pigeon

Josef Leibetseder Poster Award - In remembrance of the late Prof. Josef Leibetseder.

This is a prestigious over-arching ESVCN Award

Josefa Madrid *et al*.: Effect of the type of feed on the growth and composition of *Zoophobas morio*

ESVCN/WALTHAM Student Award - Best oral presentation on a companion animal topic

WALTHAM Petcare Science Institute

Caitlin Grant *et al*.: Metabolomic signature of energy restriction in obese cats

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Cat Nutrition Award sponsored by Nestlé-Purina.

Best oral presentations in the field of cat nutrition.

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Guido Bosch *et al*.: Fermentability of undigested residues from insects in cats: an in vitro pilot study Unfortunately Guido was not present to receive his prize

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Comparative Nutrition Award sponsored by Dechra-Specific

Best oral presentations in comparative nutrition.

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Linda Böswald et al.: Investigations on dietary and faecal Ca/P ratios in hindgut and foregut fermenters

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Best Video Poster Presentation: Giarda Morelli et al., A survey on domestic commercial dry pet food storage practices

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Giulia Giordani Poultry Nutrition Awards: Local Organising Committee Award in remembrance of the late Prof. Giulia Giordani - Best Posters in Poultry Nutrition

- Effects of the administration of a symbiotic preparation on productive performance and plasma metabolomics profile of broiler chickens. *M. Zampiga*[7]*, L. Laghi, B. Syed and F. Sirri*
- An overview of dietary effects of *Hermetia illucens* meal in Muscovy duck. *M. Gariglio*[8]*, S. Dabbou, I. Biasato, F. Gai, E. Colombino, M. T. Capucchio, A. Trocino, L. Gasco and A. Schiavone*
- Influence of dietary fat source on productive performance and egg internal quality during early egg production. *M. Palomar-Lloris*[9]*, M. D. Soler, P. Pedrola, O. Piquer, J. A. García-Bautista and C. Garcés-Narro*

CONGRESS PROGRAMME

THURSDAY 17th September

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17:15	11:15	23:15	35	Fractures in a hand raised European hare (Lepus europaeus): a case report
17:30	11:30	23:30	44	Towards the determination of fibrous matter: the importance of analysing both plant and animal fibre
17:45	11:45	23:45	127	Effects of age at induction of exocrine pancreatic insufficiency (EPI) on bone mineral density – preliminary results of a study performed in pancreatic duct ligated pigs
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18:15	**12:15**	**00:15**		**Break**
				Session 2A Minerals Chair: Annette Liesegang
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19:15	13:15	01:15	111	Effects of diets differing in level of dietary cation-anion difference and calcium concentration on renal and gastrointestinal calcium homeostasis in goats
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17:30	11:30	23:30	107	Nutritional management of muscle atrophy in a horse: a Case Report
17:45	11:45	23:45	30	Diabetes mellitus and adverse food reaction in a dog – case report
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18:15	**12:15**	**00:15**		**Break**
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19:00	13:00	01:00	71	Elderly obese cats require lower energy and higher protein intake to maintain the rate of weight loss
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19:30	**13:30**	**01:30**		**Break**
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16:45	10:45	22:45	13	Diversity of the faecal microbiota of sows before and after farrowing in 20 different farms in Germany	**16:25**	10:25	22:25	93	Phytogenic feed additive reduced ammonia and odor emissions in weaned piglets	**16:25**	10:25	22:25	80	Quantification of fecal short chain fatty acids in lean and obese dogs: preliminary results
16:50	10:50	22:50	36	Effect of diet transition and duration on a high-grain ration on faecal pH and particle size distribution in dairy cows	**16:30**	10:30	22:30	49	Apparent digestion of total and highly soluble phosphate in adult dogs fed different phosphate sources	**16:30**	10:30	22:30	106	Female and mixed breed dogs have greater difficulty for weight loss
					16:45	10:45	22:45	55	A long term feeding trial to aid in establishing No Observed Adverse Effect Levels (NOAELs) for different sources of phosphorus in feline diets					
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17:45	11:45	23:45	56	Effects of increasing rye levels in compound feeds on fermentative processes in the alimentary tract of young pigs	**17:45**	11:45	23:45	48	Environmental impact of commercial diets for dogs in Brazil	**17:35**	11:35	23:35	121	Modified gene expression in visceral adipose tissue of young Beagle dogs is associated with adult obesity
17:50	11:50	23:50	108	Effect of type of cereals (wheat and wheat with rye) on fermentation processes determined in-vitro in canine model	**17:50**	11:50	23:50	119	Owner perceptions of health for dogs fed plant-based or meat-based diets	**17:40**	11:40	23:40	73	Evaluation of the nutrient supply of prescription diets for obese dogs available in the brazilian market
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20:15	14:15	02:15	1	Leukoencephalomyelopathy in cats	**20:15**	14:15	02:15	14	Determining praecaecal, postileal and total tract digestibility of rye based diets when substituting soybean meal with rapeseed meal in Göttingen minipigs
					20:20	14:20	02:20	17	Survey: puppy feeding practices and perceptions in Switzerland
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CET/ Munich	US/EST	Hong Kong	No.	SATURDAY 19th September STREAM 6
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14:30	08:30	20:30	129	Diet Can Shift the Changes of Gut Microbiota of Dogs Closer to Wolves
14:35	08:35	20:35	9	Mixed fungal-bacterial inoculum on alfalfa (Medicago sativa L.) crop: effects on forage proximate and fatty acid compositions
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15:30	09:30	21:30	72	Nutritional management of dogs with heart disease: the owner's behavior
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17:30	11:30	23:30		Cat Nutrition Award
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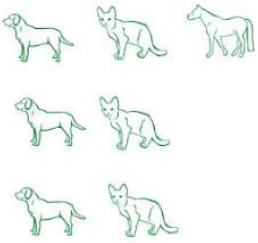

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*catalogue of feed materials - Commission Regulation (EU) No 68/ 2013 of 16 January 2013 and amendments.

ABSTRACTS & LINKS

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ABSTRACTS

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American Academy of Veterinary Nutrition
Award Winner

Evaluation of dog owners' feeding habits and influence of specific hygiene protocols

E. Luisana
Veterinary Clinical Nutrition Resident at North Carolina State University-College of Veterinary Medicine email: ektownse@gmail.com

Introduction

In-home pet food handling and food bowl hygiene practices can have adverse health impacts for both humans and pets. Safe food and bowl handling guidelines are not easily evidenced for pet owners. The study was designed to investigate dog owners' feeding habits; and evaluate the impact of FDA hygiene protocols on dog food bowl contamination.

Materials and methods

Procedures and surveys were approved by NCS-IACUC and -IRB. Pet feeding and food bowl hygiene data were collected from 406 dog owner surveys and 70 food bowl swabs. Total aerobic cell counts (TAC) were performed on 70 bowls and randomly assigned into Group A (FDA pet food handling guidelines), Group B (FDA human food guidelines), or Group C (no guidelines). Hygiene protocols were instituted in-home for 1 week, followed by a second TAC and follow-up survey.

Results

Initial survey from dog owners-households indicated: 4.7% were aware of FDA guidelines; 35% have individuals ≤12 years old and/or immunocompromised; 44% store dog food 0-5 feet from human food; 38% wash their hands after feeding; and 32% prepare their dog food on human food preparation surfaces.

Groups A and B exhibited decreases (P=<0.001, P=0.026, respectively) in bowl cell counts following hygiene protocols, as compared to Group C (P=0.373). Hot water (>160F) washing decreased TAC (P=0.005) over cold/lukewarm water. In the follow-up survey, 8% of Group A and B respondents reported likely to adhere to protocols long-term.

Conclusion

This study suggests a need for pet food handling guideline education to minimize bacterial contamination of bowls, especially in high risk households.

Session 1A Comparative nutrition

Chair: Britta Dobenecker

Feeding of bakery by-products as a substitute for grains improves apparent total tract digestibility and increases concentrations of fecal SCFA associated with a reduction in fecal pH

Kaltenegger A, Zebeli Q
University of Veterinary Medicine, Institute of Animal Nutrition and Functional Plant Compounds,
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Introduction: Leftover bakery by-products (BP) may serve as alternative to human-edible cereal grains in diets of dairy. BP could be a valuable source of energy and, compared to grains, contains less starch and NDF, but more fat and sugars [1]. The effects of heat exposure during baking are likely to affect total tract digestibility and fermentation in hindgut [2]. The aim of this study was to evaluate the effect of graded substitution of grains by BP on the digestibility of nutrients as well as on fecal short chain fatty acid patterns and fecal pH.

Animals, materials and methods: Twenty-four lactating Simmental cows (149±22.3 days in milk, mean lactation number: 2.63±1.38, initial body weight: 756±89.6 kg, 29.6±0.32 kg energy-corrected milk at the start of the trial) were fed a total-mixed ration containing 50% concentrates, 25% maize silage and 25% grass silage (dry matter-basis) throughout the experiment. The first week was considered as baseline, in which all cows received a control diet (without BP). During a 14d adaptation followed by a 14d sampling period cows were allocated intro three groups differing in their BP-concentrations of diets. 1: CON (0% BP, 30% grains), 2: 15% BP (15% BP, 15% grains), 3: 30% BP (30% BP, 0% grains). Fecal grab samples were taken from the rectum at the end of baseline (d0), and on d14 and d28 shortly before morning feeding. The pH of the fresh feces was measured by a portable pH meter and concentrations of fecal SCFA were then analyzed in the lab. Fecal samples for determining apparent total tract digestibility (ATTD) by using acid insoluble ash as marker [3] were collected twice a day on four consecutive days during baseline, adaptation and sampling period. Statistical analysis was conducted using the MIXED procedure of SAS, considering measurements carried out during baseline as covariates and including repeated measurements.

Results and discussion: The dry matter intake and milk yield linearly increased by feeding BP ($P<0.01$). Analysis of SCFA in feces revealed a linear increase of total SCFA concentration in cows fed BP compared to CON ($P<0.01$). In cows of 30% BP-group a significant higher proportion of butyrate compared to the other groups was calculated ($P<0.05$). The fecal pH linearly decreased by enhancing the inclusion of BP ($P<0.01$). The higher concentrations of fecal SCFA suggests that nutrients of BP-diets resist the microbial degradation in the rumen, which increases hindgut fermentation, resulting in a decrease in fecal pH. The ATTD of all nutrients, except from non-fiber carbohydrates and CP, increased in cows fed BP in a linear fashion ($P\leq0.05$).

Conclusion: Results suggest that BP provides a higher proportion of ruminal resistant nutrients and that the replacement of grains by BP improves the utilization of supplied nutrients in high-yielding dairy cows.

References: [1] Humer et al. (2018), [2] Ljøkjel et al. (2003), [3] Huhtanen et al. (1994)
Acknowledgments: The authors acknowledge Königshofer Futtermittel for providing the concentrates and the H. Wilhelm Schaumann Stiftung for financial support.

Fractures in a hand raised European hare (*Lepus europaeus*) – a case report

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Introduction. European hares (*Lepus europaeus*) are native to Switzerland and are often cared for by the "Stiftung Wildstation Landshut", a non-profit wildlife rehabilitation center in Switzerland. The main reasons for intake are predator attacks (mainly cats and dogs) as well as unintentional abduction by members of the public who belief them orphaned. As hares are precocial, the leverets are born above ground and mothers visit them only once a day for 2–3 min for suckling for roughly four weeks, when weaning occurs [1]. Leverets increase their body weight by a factor of 8.5 during 35 days with a maximum relative growth rate within the 1st week of life [2]. These unique adaptations are the reason that the hand raising of leverets is extremely challenging with regards to nutrition. **Animals, material and methods.** A female European hare leveret, 220 g body weight, estimated age 7 days, was fed 3 times daily with a milk replacer up to a weight of 260 g, then decreased to twice daily up to a weight of 400 g, and then the milk replacer was offered and consumed from a bowl. To imitate hare doe milk, the milk replacer consisted of the following: 1 part Royal Canin Babycat milk powder diluted with 2 parts fennel tea. This solution was supplemented with 10% of lactose-free cream (35% fat) and 1% Korvimin ZVT reptile (Table 1). In addition to the milk replacer, the leveret received soil and water in a bowl as well as fresh greens (different species of grasses and herbs, mainly Taraxacum ssp and Plantago ssp). Hard pellets of mountain hares (*Lepus timidus*) from a nearby zoo were offered as natural probiotic. **Results and discussion.** The daily weight gain of the leveret ranged between -15 g (day of arrival) and 52 g (day of release, DOR). At DOR, the hare weighed 897 g and was to be released at the place it was originally found. On the way to the release, the hare fractured both hindlimbs and had to be humanely euthanized. Further results of the analysis regarding bone health are currently pending. Differential diagnoses include nutritional causes (missing UV light, low phosphorus content of milk replacer, wide Ca:P ratio) as well as stress and trauma during the transport to the release site.

Table 1. Declared dry matter (DM), crude protein (CP), ether extract (EE), Lactose, Calcium (Ca), Phosphorus (P) and Calcium: Phosphorus Ratio (Ca:P) of the milk replacer used compared to the milk of the European hare (all original matter).

	DM [%]	CP [%]	EE [%]	Lactose [%]	Ca [%]	P [%]	Ca:P
Royal Canin Babycat	97	33	39	19	1.1	0.8	1.35
Royal Canin Babycat + fennel tea (1:2)	32.3	11	13	6.3	0.37	0.27	1.35
Royal Canin Babycat + fennel tea (1:2) + 10% lactose- free cream	32.3	11.3	16.5	6.4	0.37	0.27	1.35
Royal Canin Babycat + fennel tea (1:2) + 10% lactose-free cream + 1% Korvimin ZVT	32.3	11.3	16.5	6.4	0.51	0.34	1.5
Milk of the European hare [3]	32.5	10	15.5	1.5	0.49	0.41	1.2

Conclusion. Hand raising brown hares is nutritionally very challenging.

References: [1] Broekhuizen et al. (1980). J ZOOL, 191(4), 487-501. [2] Hackländer et al. (2002). J COMP PHYSIOL B, 172(2), 183-190. [3] Lhuillery et al. (1984). J COMP PHYSIOL A, 78(1), 73-76.

Towards the determination of fibrous matter: the importance of analysing both plant and animal fibre

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Introduction. Animal fibre is the poorly and non-digestible fraction of non-muscle carcass tissues of prey animals. It mainly consists of nitrogen-rich and protein-based molecules. The common techniques used for fibre analysis were developed for fibres derived from plants, which are carbohydrate-based molecules, and are not designed to analyse fibrous components present in animal-based foods. Animal fibre may have putative gastrointestinal and systemic health effects as shown in cheetahs[1]; hence, the development of techniques that quantify both non-digestible carbohydrates and proteins are important.

Materials and methods. The Fibrous Matter (FM) method is based on the *in vitro* digestion of Hervera et al[2]. Piglet feed and raw chicks were utilized. Tendon was analysed separately to determine the recovery of this tissue. All incubations were done with 0.5 g of defatted and dried samples. Additionally, piglet feed was spiked with 0.05 g insoluble and 0.10 g soluble plant fibre (cellulose (C) and pectin (P)) and 0.10 g insoluble and soluble animal fibre (hair (H) and tendon (T)). The FM method mimics the *in vivo* carnivorous gastric digestion (step 1; pepsin, pH 2, 2h incubation at 39°C) and the small intestinal digestion (step 2; pancreatin, pH 6.8, 4h incubation at 39°C). The digested samples were centrifuged twice, with discarding of the supernatant and addition of demineralized water. The final centrifugate was dried (overnight at 105°C), weighed, ashed (4h at 550°C) and weighed. Fibre (%) was calculated as $[(R_{SAMPLE} - A_{SAMPLE} - B)/SW] \times 100$ where R = residue weight (mg), A = ash weight (mg), $B = R_{BLANK} - A_{BLANK}$ and SW = sample weight (mg). All spiked incubations and incubations with tendon were done in triplicate. The other incubations were done in quadruplicate.

Results and discussion. Table 1: *In vitro* dry and organic matter digestibility of sample (%; dDM, dOM), fibre content of sample (% of DM), coefficient of variation of fibre analysis (%, CV) and spiking recovery (%; SR).

	Tendon	Piglet feed	Piglet feed				Raw chicks
			C	P	H	T	
dDM	98.6	62.5	53.9	61.4	42.5	62.4	59.6
dOM	98.8	68.0	58.7	66.4	47.0	66.5	70.3
Fibre	1.2	32.0	37.5	27.9	44.1	27.8	29.7
CV fibre	15.9	6.1	6.4	2.6	3.1	5.2	4.7
SR			86.7	11.3	92.6	10.8	

C, cellulose; P, pectin; H, hair; T, tendon; dDM, dry matter digestibility; dOM, organic matter digestibility; CV, coefficient of variation; SR, spiking recovery.

Tendon appeared highly digestible due to solubilisation of collagen, which is in line with other studies[3,4]. When spiking with insoluble fibre, there was a moderate recovery (SR < 95%). A dose response study is warranted to investigate the linearity of recovery for the FM method. Only a small amount of soluble fibre was recovered, which relates to removal of these fibres in the supernatant. Except for tendon, the FM method showed a moderate precision (CV < 10%). Because this method is based on the carnivorous digestive system, with their respective digestive enzymes, the dDM and dOM of piglet feed is low.

Conclusion. Considering the moderate recovery and precision, the FM method needs to be further optimized to quantify insoluble plant and animal fibre. To quantify soluble fibre, further analysis on the supernatant is required.

References: 1-Depauw *et al.* (2013) J. Anim. Physiol. Anim. Nutr. 97, 146–154; 2-Hervera *et al.* (2009) Anim. Physiol. And N. 93, 366-372; 3-Reuterswärd (1985) J. Food Technol. 20, 129-143; 4-Reuterswärd & Fabiansson (1985) J. Food Sci. 50, 1523-1525.

Effects of age at induction of exocrine pancreatic insufficiency (EPI) on bone mineral density – preliminary results of a study performed in pancreatic duct ligated pigs

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Introduction: EPI is present in animals as well as humans, resulting in maldigestion and malabsorption. In a former study (1) in young pigs (7 weeks (wks) old) with induced EPI by pancreatic duct ligation (PL) it was shown that EPI causes reduced bone mineral density (BMD). This study aimed to test, whether induction of EPI in older pigs (aged 16 weeks) results also in reduced BMD. Data of some animals are not available yet – therefore data are presented as preliminary.

Animals, materials and methods: The study was approved by the Ethics Committee on Animal Welfare of the Hannover District Government. In some pigs the EPI was induced at the age of 7 wks (PL-7), while in a second group the pigs underwent surgery at age of 16 wks (PL-16), in control pigs (Con) a sham OP was performed at age of 7 wks. All animals were fed a compound feed and housed under same conditions. No pancreatic enzyme therapy (PERT) was given to provoke maximal effects. All pigs were euthanized at the age of 26 wks and left tibia was investigated using pqCT.

Results and discussion: BMC differed significantly between Con and PL-7 (table 1). In PL-16 values were intermediate – neither differing from controls nor PL-7. At diaphysis there were no significant differences between groups (result not shown).
Table 1: Bone mineral content (BMC) of the tibia of controls, PL-7 and PL-16 at the age of 26 wks

Localisation	Parameter	Control (n=5)	PL-7 (n=4)	PL-16 (n=3)
prox. epiphisis	BMC (mg/cm)	415 ± 29.1a	360 ± 24.1b	388 ± 38.6ab
Distal epiphisis	BMC (mg/cm)	590 ± 21.0a	464 ± 33.6b	514 ± 59.0ab

Significant differences between groups are marked by different upper letters, $p < 0,05$, t-test

Conclusion: Although duration of EPI was only 10 weeks in PL-16 and main growth period was already completed when pigs underwent surgery there was a trend for lower BMD. From these results it can be concluded that EPI affects BMD not only in very young individuals, Further investigations are needed but it seems that BMD should be checked also in adults with long lasting EPI.

References: 1) Mößeler et al. (2013): Proc. Soc. Nutr. Phys. (22), page 55 2) Sermet-Gaudelus et al. (2011): J. Cystic Fibrosis, 10 Suppl 2, S16–S23

Session 2A Minerals I

Chair: Annette Liesegang

Concentrations of total and highly soluble phosphate in commercial renal diets for cats and dogs purchased in Germany

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Introduction: The concentration of highly soluble phosphates (P_{sol1}), i.e. soluble in water within one minute, has been analysed in various processed cat and dog foods marketed in Germany[1]. High solubility is a precondition for P absorption from the gastro-intestinal tract [2] and therefore linked to potentially harmful health effects[3,4,5]. A high concentration of total P (P_{total}) as well as P_{sol1} was found in these products. Diets for renal patients should contain limited amounts of P. The aim of the actual study was to evaluate the concentration of total and highly soluble P in processed renal diets for cats and dogs marketed in Germany. **Material & methods**: Commercial processed renal diets for dogs and cats were purchased. To this date, the sample pool consists of 39 products (20 moist and 15 dry renal diets for cats, 4 dry renal diets for dogs). After measuring the amount of P_{total} (photometric, modified vanadate molybdate method[5]), the fraction of soluble P (P_{sol1}, after 1 minute in water) was determined using the method described by Lineva et al.[6]. The amount of P_{sol1} and P_{total}, respectively, was then related to energy (MJ ME[7]). **Results**: The concentration of P_{total} was above maintenance requirements[8] in all analysed samples (range: 194-1137 mg P/MJ or 1.2-7.1 times the requirements (cats: 160 mg P/MJ ME; dogs: 180 mg P/MJ ME)). In 56.4% of the tested products, the concentration amounted to more than twice the recommended daily intake for healthy animals. In every fourth moist diet for cats, P requirements were already met solely by P_{sol1}. In dry renal diets for cats, this held true for every third product. The amount of P_{sol1} ranged from 44 to 357mg/MJ ME.

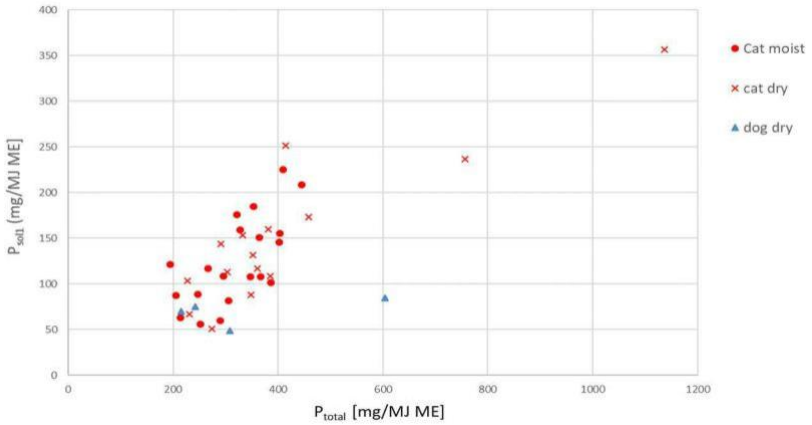

Fig. 1: Amount of soluble phosphate (after 1 minute in water; P_{sol1}) in relation to the amount of total phosphate (P_{total}) per MJ ME in renal diets for cats and dogs.

Discussion: The results of this survey demonstrate that the majority of tested samples of processed renal diets for cats and dogs supply P_{total} in excessive and not in restricted amounts, as required by most if not all renal patients. In renal diets, the concentration of highly available P_{sol1} should be reduced to a minimum. 25% of moist and 33% of dry diets for cats with chronic renal disease contained considerable amounts of these highly available phosphates, which probably does not meet the customers' expectations.

References: [1]Dobenecker 2019; [2]Lineva et al. 2018; [3]Dobenecker et al. 2017; [4]Dobenecker et al. 2018; [5]Herbst, Dobenecker 2018; [6]Gericke & Kurmies (1952); [7]European standard EN16967:2017; [8]NRC 2006

GO TO ABSTRACT LIST

Correlation between faecal calcium and phosphorus excretion in adult dogs fed high phosphorus diets

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Introduction: A meta-analysis in carnivores has shown a strong linear correlation between faecal calcium (Ca) and phosphorus (P) excretion[1]. The hypothesis to explain this is that Ca and P are absorbed in a rather fixed ration of ~2:1, which enables storage of both minerals in bone. Bone turnover is thought to be the major regulating mechanism of Ca and P homeostasis in carnivores. The aim of the present study was to investigate whether the use of different P sources alters the relationship be-tween faecal Ca and P excretion in dogs.

Materials & methods: Data from several balance trials in adult Beagles[2,3] were used. Control diets were formulated with P from organic sources (tripe, rice) at maintenance levels. High P (HP) diets contained 5x the recommended daily allowance[4] from either organic sources (oP; bone meal, poultry meal) or P salts (Pi) (KH_2PO_4, $K_4P_2O_7$, NaH_2PO_4, $Ca(H_2PO_4)_2$, $CaHPO_4$, $Na_5P_3O_{10}$). Faecal Ca excretion (x-axis) and faecal P excretion (y-axis) were correlated (both in mg/kg body weight (BW)). The regression lines of oP and Pi diets were compared using the test of Ho (p=0.05).

Results & Discussion: In all diets with organic P sources (control, HP bone meal, HP poultry meal), there was a strong linear correlation between faecal Ca and faecal P excretion (Fig. 1, R^2 = 0.97), which is in accordance with previous data on carnivores[1] while in the HP diets with Pi, however, the relationship was remarkably weak (R^2 = 0.30). There was a significant difference between the slopes of the regression lines of oP and Pi diets (p<0.001). The lower slope in the Pi diets indicates a lower faecal P excretion in relation to Ca excretion than in the oP diets. We hypothesise that this is due to higher availability of Pi sources, which is also reflected by serum P kinetics[3]. Dietary Pi is not bound to Ca, unlike some oP compounds. Possibly, absorption of highly soluble P salts happens early in the gastrointestinal tract of dogs mostly via passive mechanisms, before Ca-P-complex formation[1] can reduce availability.

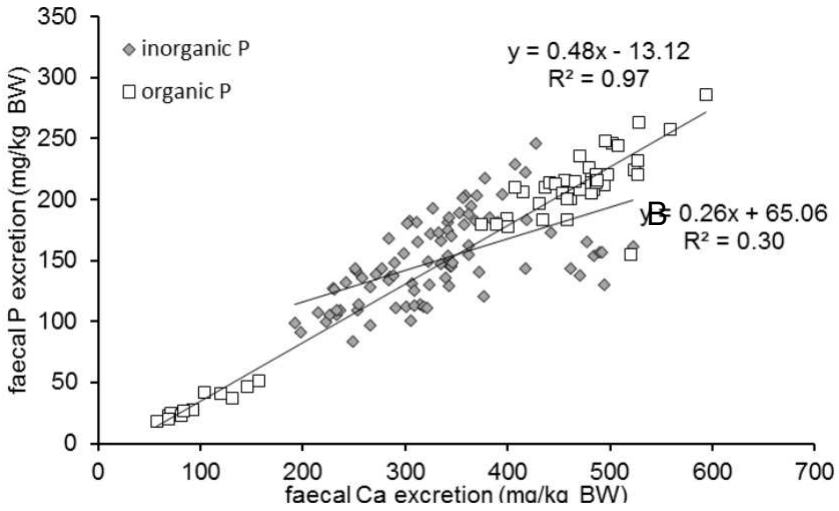

Fig. 1: Correlation between faecal Ca and P excretion [mg/kg BW] in dogs fed HP diets with P salts or control diets with organic P sources.

Conclusion: The otherwise strong linear correlation between faecal Ca and faecal P excretion seems to be disrupted by Pi intake in dogs.

References:
[1]Böswald et al. (2018) J Anim Physiol Anim Nutr, 102(2), 370-379; [2]Siedler (2018) Doctoral thesis, LMU München; [3]Herbst, Reese & Dobenecker (2020) submitted; [4]NRC (2006) Nutrient Requirements of Dogs and Cats. National Academic Press, Washington D.C.

GO TO ABSTRACT LIST

Inorganic P salts disrupt the strong linear correlation between faecal calcium and phosphorus excretion in dogs but not in cats

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Introduction: In carnivores, a strong linear correlation between faecal calcium (Ca) and phosphorus (P) excretion has been shown[1]. In dogs, highly soluble P salts in the diet seem to disturb the linear correlation of faecal Ca and P excretion[2]. The aim of the present work was to explore how high P (HP) diets using inorganic P sources (Pi) alter the correlation in cats.

Materials & methods: Data from several balance trials (control and HP diets) in cats[3,4,5] was used. The experimental diets contained P from organic sources (oP; meat, carbohydrates, bone meal, poultry meal) and in HP diets additionally Pi (KH_2PO_4, $K_4P_2O_7$, NaH_2PO_4, $Ca(H_2PO_4)_2$, $CaHPO_4$, $Na_5P_3O_{10}$). Data was grouped according to P source (Pi vs. oP). Regression analysis of faecal Ca excretion (x-axis) and faecal P excretion (y-axis) was conducted in the unit mg/kg body weight (BW). The regression lines were compared with the test of Ho ($p<0.05$).

Results & Discussion: In cats, the linear relationship between faecal Ca and P excretion was extremely strong (Fig. 1). In contrast to dogs[2], there was no significant difference between the Pi diets and oP diets ($p=0.365$). There was also no significant difference between the regression lines when grouped according to dietary Ca/P ratio (dietary Ca/P<1: $y=0.63x+14.9$, $R^2=0.98$; dietary Ca/P≥1: $y=0.64x-11.01$, $R^2=0.97$; $p=0.793$). These findings imply that dogs and cats show a similarly strong linear correlation of faecal Ca and faecal P when fed diets with P from organic sources.

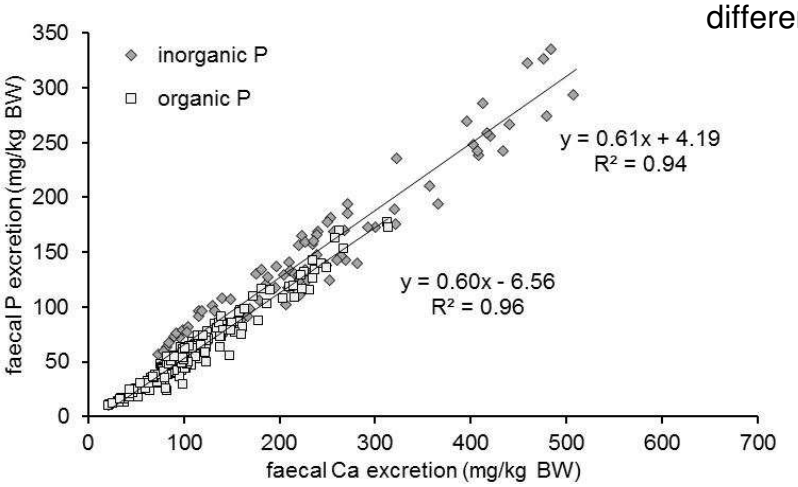

Fig. 1: Correlation between faecal Ca and P excretion [mg/kg BW] in cats fed HP diets with P salts or control diets with organic P sources.

Conclusion: In cats, Pi sources did not disrupt the linear correlation between faecal Ca and P excretion, as it was the case in dogs. There may be species differences in Ca and P handling between these carnivorous species that are not yet completely understood.

References:
[1]Böswald et al. (2018) J Anim Physiol Anim Nutr, 102(2), 370-379; [2]Böswald et al. (2020) Proceedings of the Society of Nutrition Physiology Conference, Göttingen, Germany; [3]Demmel (2011) Doctoral thesis, LMU München; [4]Hertel-Böhnke (2018) Doctoral thesis, LMU München; [5] unpublished.

GO TO ABSTRACT LIST

Effects of diets varying in level of dietary cation-anion difference and calcium concentration on renal and gastrointestinal calcium homeostasis in goats

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Introduction: Recent studies showed that a low dietary cation-anion difference (low DCAD), may reduce the incidence of milk fever in ruminants compared to high DCAD[1-2]. By modulating the body's acid-base balance towards a moderate metabolic acidosis, a low DCAD enhances the calcium (Ca) resorption from bones and the renal production of 1,25-(OH)2D. In addition, it is proposed that the lower pH leads to a better responsiveness of the target tissues. The details of the mechanisms are still unclear. In this experiment, basic effects were studied in a goat model. The hypothesis was that acidifying the diet leads to an upregulation of intestinal calcium transport and Vitamin D receptors in goats and additionally to changes in excretion of Ca via the kidneys

Animals, material and methods: 24 Saanen kids aged 6 months were divided into 4 feeding groups (HL (high DCAD/low Ca), LN (low DCAD/normal Ca), LL (low DCAD/low Ca) and HN (high DCAD/normal Ca)). The effective amount of anion and cation salts in the ration for each group was calculated and controlled by monitoring the urinary pH. The DCAD level (mEq/kg dry matter (DM)) was -141 (low) and 142 (high). The Ca content in the diet (g/kg DM) was 2.8 (low) and 4.6 (high). On day 0 the metatarsal bone density was measured in all animals by peripheral quantitative computed tomography. Urine, serum and faecal samples were taken on day 0, 4, 7, 14 and 22. On day 22 animals were slaughtered. Samples of the rumen fluid and of the organs involved in the absorption and metabolism of Ca (rumen, duodenum, kidneys, parathyroid, metatarsal bones) were collected.

Results and discussion: The diet did not affect the weight gain of the animals (average 130 g/day). The mean urinary pH values (±standard deviation) for each group were as follows: HL 8.2 ±0.1, LN 6.8 ±0.2, LL 7.0 ±0.4, HN 8.3 ±0.1. The urinary Ca excretion (mg/l) in the groups LN (564 ±200) and LL (208 ±135) exceeded those of to the groups HL (37 ±12) and HN (51 ±15). Serum Ca levels (mg/l) did not differ between groups: HL 79.3 ±1.8, LN 87.7 ±1.3, LL 79.3 ±0.8, HL 85.3 ±0.8. For each sampling point the values (min. and max.) of osteocalcin (43-155 ng/ml), 1,25-(OH)2D (89-235 pmol/L) and of the biochemical markers for bone turnover (serum CrossLaps 0.6-2.2 ng/ml) increased progressively in all groups. The values of 25(OH)D ranged from 60 to 105 nmol/l. On day 22 the serum iCa levels showed no difference between groups (iCa mmol/l): HL 1.2 ±0.13, LN 1.3 ±0.07, LL 1.2 ±0.10, HL 1.2 ± 0.09. Ca levels (mg/l) in rumen fluid were higher in groups with a dietary calcium restriction (HL 101 ±37, LL 108 ±0.31) than in those with an adequate intake (LN 61 ±18, HN 83 ±34). The rumen fluid pH ranged from 5.3 to 6.8.

Conclusion: The serum levels for iCa and Ca remained constant with the different contents of Ca and DCAD levels in the diets, which is not astonishing due to homeostatic mechanisms. The urinary Ca excretion in the groups with a low urinary pH exceeded that of the groups with a higher urinary pH. However, the effect of the use of acidifying salts in diets have to be investigated in detail, especially regarding the influence on renal calcium transport and Vitamin D receptors.[3-6].

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Vinegar test as a simple method to assess the availability of Magnesium in supplemental sources

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Introduction: Magnesium (Mg) is an important macromineral that has many functions within the body. Farm animals rely on a continuous dietary supply of absorbable Mg and supplemental sources are commonly used in rations to ensure adequate Mg supply. However, there are distinct differences in the availability of Mg from a large list of choices for supplemental Mg. Due to the increasing number and origin of sources of feed grade Mg, there is a need for a relatively quick laboratory method that could provide a reasonably accurate value of apparent availability of each source that could be used in diet formulation. Vinegar test was proposed as a simple method to differentiate the reactivity and potential alkalizing properties of Mg oxide (MgO) sources (1). The present study evaluated the usefulness of vinegar test to determine the availability of Mg in various supplemental sources.

Material and methods: In total, 14 different Mg products being the source of MgO, Mg sulfate, Mg acetate, or Mg phosphate were included for the test. As analysed, the products varied in the content of Mg (11 – 56%). For the vinegar test, precisely 3.0 g of each product was placed in a container and 40 mL of 5% acetic acid solution was slowly added to the product. The sample was mixed for 15 s, let sit and mixed again after 15 min-mark. The pH of the solution was measured at 30 min, 3 h, 24 h, and 48 h after adding the acetic acid solution. At each time point, the solution was taken for analysis of the Mg content using inductively coupled plasma optical emission spectroscopy (ICP-OES, Avio® 500, PerkinElmer, MA, USA). The solubility (%) was estimated as followed: the soluble Mg content / the Mg content of original product × 100. Data on the pH and Mg content were subjected to a regression analysis using Proc Reg of SAS. The fixed effect of time on pH and the solubility was analysed using Proc GLM of SAS considering the random effect of products in the model.

Results and Discussion: Of the 14 products, 4 products: Mg sulfate, Mg acetate, clinoptilolite and one MgO product were detected as outliers and removed from the regression analysis. For these outliers, both Mg acetate and Mg sulfate were readily soluble but had low alkalizing potential, while the solute of the MgO product had very high alkalizing properties. For the remaining data (n = 67), the pH of solution of these data ranged from 3.55 – 5.59 and the solubility from 5 – 47% and there was a strong linear relationship between pH and soluble Mg content with the following prediction equation: Mg (g/kg) = 56 (SE = 3.2) × pH -194 (SE = 15) + ($R2$ = 0.821, P<0.001, RMSE [2]. 13.9). There was a time effect on pH and solubility of Mg. The solubility increased with incubation time with the solubility at 24 and 48 h being higher than those at 30 min and 3 h (P<0.05).

Conclusion: Data suggest that the vinegar test could be a simple method for screening the availability of Mg based on both the solubility and the reactivity and potential alkalizing properties of different Mg sources, especially MgO which is the most common Mg supplementation products. However, this test is not suitable for Mg acetate and Mg sulfate. Further tests with a larger number of Mg samples and importantly in vivo data are warranted to validate the prediction equation that will have significant implications for feed plants and nutritionists particularly those that have no access to Mg analysis.

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Determination of copper and zinc concentrations in Brazilian ingredients and commercial foods for dogs and cats

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Introduction. Cu levels in pet food may be inadequate. The soil contamination, due to the use of swine wastewater as an organic fertilizer and the use of cupric fungicides, in addition to antifungals may contaminate the ingredients used by the pet food industry. An increase in liver Cu concentrations has been demonstrated over 30 years in Labrador retrievers with and without chronic hepatitis (CH) [1], and it can be related to dietary levels of this element, and to dietary levels of zinc (Zn). Therefore, the objective of this study was to determine the levels of Cu and Zn in commercial pet food, as well as in ingredients.

Animals, material and methods. A total of 100 commercial foods for dogs and cats were analyzed, 75 dry foods (DF) (61 for dogs and 14 for cats) and 25 wet foods (WF) (14 for cats and 11 for dogs), all complete and balanced. Samples of 82 ingredients (sources of protein, carbohydrates and minerals) were also analyzed. The dry matter was carried out according to AOAC [2]. Cu and Zn analyses were performed by optical emission spectrometry with inductively coupled plasma (ICP-OES). Comparisons were made between DF and WF, and between different sources of protein and carbohydrates using the SAS software ($P<0.05$), and on dry matter basis. Comparisons were made between the levels found in foods and ingredients with the FEDIAF (2019) [3] recommendations, descriptively.

Results and discussion. The legal limit (LL) for Cu expressed in FEDIAF (2019) was exceeded by 20.83% and 28.57%; and for Zn in 26.39 and 14.29% for dog and cat foods, respectively. DF showed higher levels of Cu and Zn than WF ($P<0.05$). Wheat bran (WB) was the source of carbohydrates with the highest levels of these elements and, among protein sources, meat meal (MM) and fish meal (FM) showed higher levels of Cu and Zn ($P <0.05$), respectively. The sample of dicalcium phosphate (DCF) showed Cu levels 18.04 times above the LL. Excessive Cu intake and low dietary Zn:Cu ratio are associated with an increased risk of CH in Labrador retrievers [4]. These authors observed that a group of dogs that consumed a diet with Zn:Cu ratio between 8.9 to 18.8 had higher hepatic Cu levels, compared to a group of dogs handled with a food with a zinc/copper ratio of 32.5. In our study, the Zn:Cu ratio in dog foods ranged from 2.04 to 14.38 in DF and from 5.09 to 12.91 in WF (Figure 1).

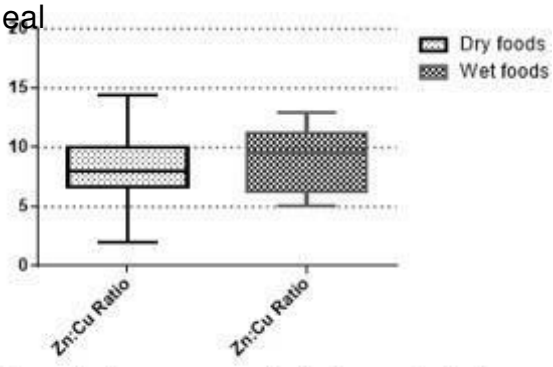

Figure 1. Zinc:copper ratio in dry and wet dog foods

Conclusion. The LL for Cu and Zn expressed in the FEDIAF (2019) were exceeded in both dog and cat foods and among the ingredients, the WB, MM and the DCF presented greater potential of contamination of Cu. According to information in the literature, the Zn:Cu ratio in the foods was low, which may imply a higher risk of CH in dogs of predisposed breeds.

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Influence of phosphorus source on the difference between dietary and faecal Ca/P ratio and serum phosphorus levels in dogs

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Introduction: Dietary phosphorus (P) sources can be grouped in "organic" P sources (oP) and inorganic phosphates (Pi). They differ in solubility[1], which is a precondition for absorption from the gastrointestinal tract. Differences in P homeostasis depending on P source were demonstrated[2]. The aim of this study was to use data from these P excess trials[2] to test whether delta, the difference between dietary and faecal calcium (Ca) / P ratios, can be used to distinguish between dietary P sources.

Animals, materials & methods: In the study of Herbst et al.[2], 8 adult Beagles (14±1 kg body weight) were fed experimental diets (64% rumen, 32% rice, 4% casein) with 5x the recommended daily allowance[3] for P from different sources. Bone meal (BM) in two particle sizes (<1mm and >1mm) and poultry meal (PM; diet composition: 57% poultry meal, 43% rice) were sources of oP and as Pi sources NaH_2PO_4, KH_2PO_4, $Ca(H_2PO_4)_2$ and $CaHPO_4$ were used. The dietary Ca/P ratio was 1.4/1 in NaH_2PO_4, KH_2PO_4 (I), $Ca(H_2PO_4)_2$ and $CaHPO_4$. In diets KH_2PO_4 (II), PM and BM, dietary Ca/P was 1.9/1. Each diet was fed for 13d of adaptation, followed by a 5d balance trial and a ≥14d washout period. On day 5 of the balance trial, blood was sampled at 8 time points and area under the curve (AUC; mmol/l*t) was calculated for serum P. In the present study, the experimental data was analysed as follows: The difference be-tween dietary Ca/P ratio and faecal Ca/P ratio was calculated (Δ) and compared be-tween diets (Holm-Sidak all pairwise test). was correlated to AUC(P).

Results & Discussion: There was no significant difference in between oP sources PM, BM <1mm and BM >1mm (means±SD: -0.27±0.09, -0.33±0.12 and -0.20±0.05, respectively). They had significantly higher values than all other diets ($p<0.001$).

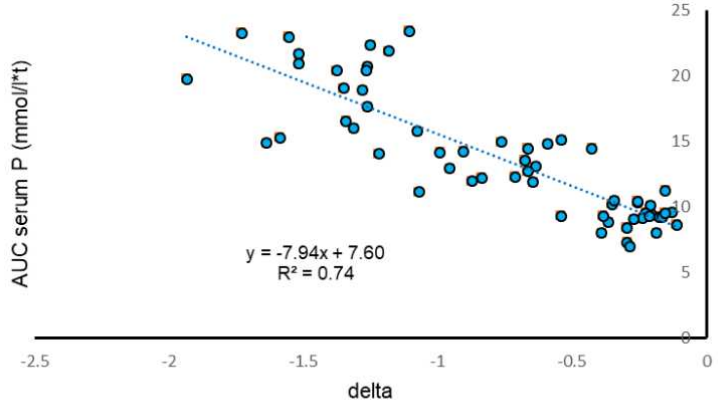

Fig. 1: Correlation between and AUC(P) [mmol/l*t] from diets with oP sources and P salts.

There was no significant difference between of NaH_2PO_4 and KH_2PO_4 at both dietary Ca/P ratios (-1.41±0.15, -1.31±0.24 and -1.40±0.25, resp.). Between and AUC(P), a strong inverse correlation (R^2=0.74) was observed (Fig. 1).

Discussion: Extremely low values were found in Pi diets, esp. in KH_2PO_4 and NaH_2PO_4, which indicates higher P availability. Diets with less soluble Ca-phosphates had significantly higher values than with highly soluble Na- and K-phosphates. The tight correlation between and AUC(P) indicates that, unlike apparent P digestibility[2], may serve as a predictor for effects on serum P and therefore P homeostasis in dogs.

Conclusion: The tested Pi sources had a higher availability, resulting in lower values than those of oP sources. Therefore, could serve as an easy to determine parameter to help distinguish P sources in pet food and their effects on the animal. The dietary Ca/P ratio did not influence in KH_2PO_4 diets.

References:

[1]Lineva et al. (2019) J Anim Physiol Anim Nutr, 103(1), 317-323; [2]Herbst, Reese & Dobenecker (2020) submitted; [3]NRC (2006) Nutrient Requirement of Dogs and Cats. Washington D.C.

This data has already been presented at the 74th GfE Conference, Göttingen.

Effects of a phosphorus-reduced diet on vitamin D metabolism in young goats

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Introduction. Feeding low phosphorus (P) diets to livestock is desirable for economic and ecological reasons. Ruminants cope more easily with P reduced diets than monogastric species due to their endogenous P circulation. Previous studies with sheep and goats fed P-reduced diets unanimously displayed significant changes in mineral homeostasis, such as hypophosphatemia and hypercalcemia [1-4]. Regarding the effects of dietary P depletion on vitamin D metabolism, these studies revealed contradictory findings: while blood 1,25-dihydroxyvitamin D3 (calcitriol) remained not affected in most studies [1-3], a significant decrease occurred in thyreoparathyroidectomized ruminants, which consequently lacked parathyroid hormone (PTH) [4]. The renal enzyme 25-hydroxyvitamin D-1 alpha hydroxylase (CYP27B1) converts 25-hydroxyvitamin D3 (calcidiol) to calcitriol, while 1,25-dihydroxyvitamin D3-24-hydroxylase (CYP24A1) inactivates both calcidiol and calcitriol. The aim of the present study was to investigate changes in mineral homeostasis influencing the modulation of vitamin D metabolism in young goats fed P-reduced diets.

Animals, material and methods. Over six weeks, young male goats, allocated into two groups of 7 animals each, received diets containing 1.10% calcium (Ca) and either 0.42% or 0.10% P. Blood samples were collected from the external jugular vein before slaughter. Kidney cortex was obtained immediately after slaughter and stored at -80°C. Blood inorganic phosphate (P_i) and Ca concentrations were determined colorimetrically by standard photometric techniques, while calcidiol and calcitriol were quantified commercially by reversed-phase HPLC and an ELISA. The expression of CYP24A1 and CYP27B1 in renal cortex tissue were determined by *q*PCR. All data were tested for normal distribution by Shapiro-Wilk test and analysed by unpaired Student's t-test.

Results and discussion. In goats receiving a low P diet, expected changes in mineral homeostasis such as reduction in blood P_i ($P<0.0001$) and increased Ca ($P<0.0001$) concentrations occurred. The elevated blood Ca levels could be the result of altered bone metabolism to compensate for the low blood P_i concentrations because bone marker was changed (data not shown). The elevated blood Ca concentrations could inhibit the synthesis of PTH from parathyroid glands, which could be the reason for the diminished CYP27B1 expression ($P<0.01$) resulting in lower calcitriol ($P<0.05$) concentrations during a dietary P restriction. The CYP24A1 mRNA was not changed while the concentration of calcidiol was elevated ($P<0.05$). Therefore, translational modification or a stimulation of CYP24A1 activity cannot be excluded.

Conclusion. During a dietary P depletion, massive changes in vitamin D metabolism occur in young goats. Therefore, severe alterations in vitamin D dependent processes like bone metabolism can be assumed.

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Labelled analytical constituents of commercial dog foods according to the main point of sale. Are there any clues as to the origin of phosphorus?

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Introduction: In our subconscious, a gradation has taken place between pet food depending on where it is sold. However, are these foods objectively different and could these differences have health consequences? Here we study the analytical constituents present on dog food labels in order to provide an objective view of the market.
Material and Methods: Only commercial adult maintenance dog food is assessed, Data comes either from the manufacturer or, in the absence of data available, from online stores or physical stores. We use the lowest price found for the largest commercial package available. The energy density for each product is calculated according to NRC 2006 method[1]. The soluble phosphorus is calculated according to Lineva et al. 2018[2]. Proteins that can come from bone materials are assumed to be about 6 times the insoluble phosphorus[3]. The brands are divided into four categories according to their main point of sale and their absence of grains: veterinary, mass retailers, online/ pet store, and online/pet store grain-free. The data are analyzed with a Kruskal-Wallis then Tukey tests. **Results and discussion:** A total of 409 dry commercial dog foods (320 with phosphorus) were evaluated. The main results are presented in table 1. In this report, grain-free products are sold exclusively in online or pet shops. All foods have phosphorus levels above the FEDIAF minimum guideline (1.16g/Mcal)[4]. The highest levels of calculated soluble phosphorus are reported in mass retail and online pet store product. In addition, the phosphorus level of these products is not correlated with their level of none-bone proteins, which can be a source of organic phosphorus. In veterinary and grain-free products, the ratio is closest to the non-bone animal by-products (80-116[5]). Thus, it can be assumed that the phosphorus content of added inorganic salt would be higher in entry-level products. This phosphorus could be harmful to the kidney health[2]. It should be noted that the phosphorus levels provided on the label might be incorrect or underestimated.
Conclusion: This study highlights the differences between products depending on where they are sold, especially with regard to phosphorus. While more research is needed to understand the implication of added inorganic phosphorus on dog kidney health, its level should be provided to the consumer.
Table 1: Analytical constituents from different dog foods (median, the first and the third quartile). Different letters in the same column indicate a statistically significant difference.

Brand's key sellers (n_{phos}/n)	Protein (g/Mcal)	NFE (g/Mcal)	Fat (g/Mcal)	P (g/Mcal)	$P_{soluble}$ (g/Mcal) Calculated[2]	$Protein_{non\text{-}bone}$ vs $P_{soluble}$ ratio	Price (€/Mcal)
Mass retail (44/69)	68^{A} [62-71]	117^{A} [103-127]	35^{A} [32-40]	2.7^{A} [2.6-3.0]	0.94^{A} [0.8-1.13]	56^{A} [51-69]	0.79^{A} [0.59-0.95]
Online/Pet store (143/168)	66^{A} [61-71]	119^{A} [107-135]	35^{A} [30-39]	2.5^{B} [2.3-2.8]	0.80^{B} [0.66-0.97]	70^{B} [85-87]	1.0^{B} [0.78-1.17]
Grain-free (99/138)	79^{B} [67-95]	93^{B} [65-110]	41^{B} [38-46]	2.7^{A} [2.4-3.1]	0.84^{AB} [0.67-1.1]	84^{C} [64-102]	1.35^{C} [1.2-1.54]
Veterinary (34/34)	72^{B} [65-84]	107^{C} [91-122]	41^{B} [33-44]	2.1^{C} [1.8-2.3]	0.73^{C} [0.51-0.79]	91^{D} [82-125]	1.19^{D} [0.96-1.43]

References: 1. National Research Council. Nutrient Requirements of Dogs and Cats (2006). 2.Lineva et al. J Anim Physiol An N 103, 317–323 (2018). 3.Shirley et al. Poultry Science 80, 626–632 (2001). 4. FEDIAF. Nutritional Guidelines (2019). 5. ANSES. CIQUAL food database (2016).

GO TO ABSTRACT LIST

Homemade versus extruded and wet commercial diets for dogs: cost comparison

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Introduction. There is a growing interest of dog and cat owners in feeding their pets with homemade diets [1,2], and although it is a topic of great importance and much debated, there are few studies in the literature that verified and compared the costs between complete homemade diets and commercial foods for dogs. The present study aimed to verify and compare the costs of homemade diets in relation to the extruded and wet commercial diets for dogs in maintenance and consuming therapeutic diets (obesity, cardiopathy, diabetes, hepatic encephalopathy, chronic kidney disease, and food hypersensitivity), an important information that impacts in the choice of food by the owners.

Animals, material and methods. The maintenance energy requirements (MER) were estimated for adult dogs of different sizes (3kg, 15kg, 30kg, and 50kg) and then the daily and monthly amounts of food intake were estimated for each type of food. The costs were calculated per day and per month, per 1000kcal of metabolizable energy of product and per kg of metabolic weight of the animal. Fourteen complete and balanced homemade diets were formulated, and in each proposed group, two diets with different protein sources were used.

Results and discussion. Under the conditions and in the period of the present study, for all comparison formats and for all evaluated variables (Figures 1 and 2), homemade diets presented a higher cost than extruded diets. In addition, commercial wet diets were more expensive than homemade or dry diets for maintenance dogs.

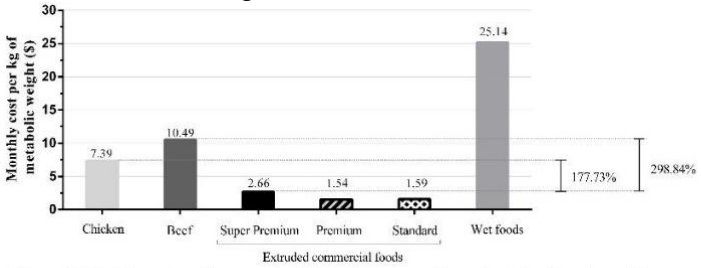

Figure 1. Monthly costs of homemade diets and commercial products for dogs in maintenance per kg of metabolic weight (kg of body weight$^{0.75}$) and relative percentage of costs between Super Premium.

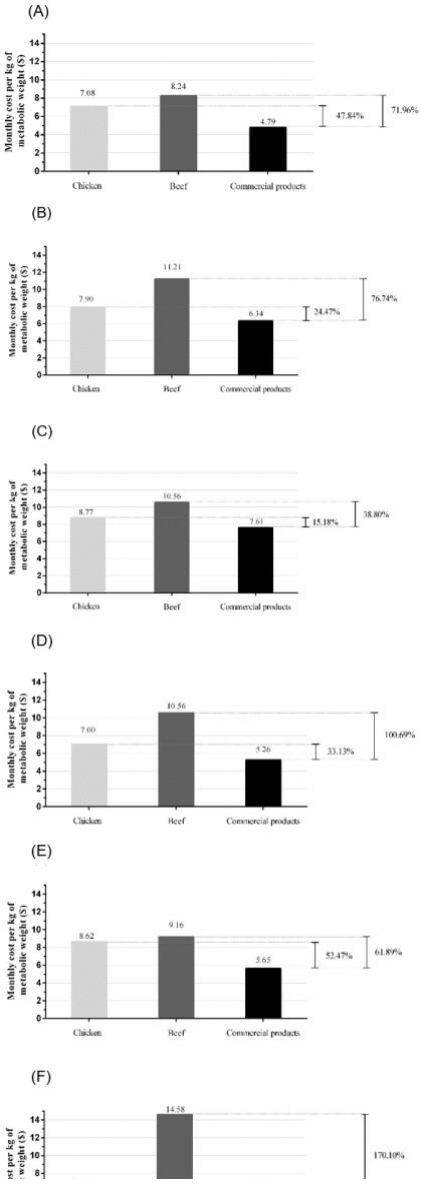

Figure 2. Monthly costs of homemade diets and commercial dry products for obesity (A), diabetes mellitus (B), chronic kidney disease (C) congestive heart failure (D), hepatic encephalopathy (E), and food hypersensitivity (F) per kg of metabolic weight (kg of body weight$^{0.75}$) and relative percentage of costs between extruded diet and both homemade diets.

Conclusion. Homemade diets were more expensive than dry maintenance and therapeutic dry diets, and commercial wet diets were more expensive in all scenarios.

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Session 1B Case reports

Chair: Linda Böswald

Effects of a home-made elimination diet and probiotics on *Helicobacter spp,* refractory infection in a series of dogs with symptomatic gastroenteropathy

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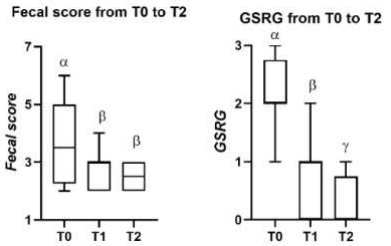
Introduction. *Helicobacter spp*. (H), a spiral Gram-negative bacterium, has been associated with gastroenteropathy and dysbiosis in dogs [1]. Currently, traditional treatment (clarithromycin/metronidazole, amoxicillin and proton-pump inhibitor, also known as "triple therapy") [2] fails in > 20% of cases, due to antimicrobial resistance and patient non-adherence [3]. This situation has encouraged the search for new approaches. In humans, some probiotic strains showed a high rate of successful eradication [4,5], and dietary management is a key factor in attenuating the pathological processes initiated by H and re-establishing eubiosis [6]. However, data in veterinary medicine are lacking. **Animals, material and methods**. Eight clinically ill dogs with chronic gastroenteropathy and *H* refractory infection [7,8] were enrolled (T0). Before study inclusion, each animal had already been fed a commercial elimination diet, received oral metronidazole (10mg/kg PO q12h), amoxicillin (22mg/kg PO q12h) and omeprazole (0.66mg/kg PO q24h) for 21 days [1]. From T0, a home-made elimination diet with a novel intact protein was prescribed [energy density (kcal EM/g) 4.2; CP 28.5%, EE 12.5%; CF 2.5%, expressed as mean values on DM basis], in association with probiotics (VSL#3®, 112 to 225×10^9 lyophilized bacteria/10 kg/q24h). At T1 (30 days) and T2 (60 days) the following parameters were recorded: body weight (BW), Body Condition Score (BCS), a 7-point scale faecal score and a 3-point Gastrointestinal Symptom Rating Scale (GSRS), modified from [9]. Furthermore, non-invasive tests were repeated to check for *H* eradication at T2. A RM One-way ANOVA with the Geisser-Greenhouse correction was performed; significant differences were considered for $p < 0.05$. **Results and discussion**. Adherence to dietary indications was excellent in all patients. No differences were detected in BW and BCS during the trial, while both faecal score and GSRG significantly decreased from T0 to T2 ($p<0.05$ and $p<0.001$ respectively, see Fig.1-2), meaning clinical resolution of gastroenteropathy. Moreover, all dogs except one tested negative to *H* at T2, suggesting infection eradication.



Conclusion. H play a role in gastric diseases in humans and dogs. This study suggests that dietary change, in addition with probiotics supplementation, may results in clinical and etiological recovery of dogs with symptomatic gastroenteropathy from refractory *H* infection, as seen in humans, likely restoring eubiosis and reducing inflammation. Nevertheless, further large-scale clinical studies are required to better understand the role played by dietary management, with or without probiotics, in *H*-associated refractory canine gastritis.

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Enteral and parenteral nutrition for a puppy with tetanus: a case report

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Introduction. Intensive care is extremely important for the treatment of tetanus patients. A negative energy balance is commonly seen due to the hypermetabolic state[1,2]. Therefore, adequate nutritional support is essential. A much debated question is whether enteral (EN) or parenteral nutrition (PN) is the best nutritional route for the management of these patients[1,2,3]. This case report assess the use of the two feeding routes for its success.

Case report. Nouba, an 11-week-old, canine, female Vizsla of 7.3kg and BCS 3/9 was referred to the Small Animal Teaching Hospital of Ghent University after been diagnosed with tetanus. Therapy consisted of antiserum, metronidazole, amoxicillin-clavulanic acid and valium. Some severe nutrition risk factors[4] were seen, such as 5 days of hyporexia, weight loss (8% BW), and difficulties to grab and swallow the food. Therefore, nutritional intervention was required and a gastric feeding tube was placed. Energy requirement calculations were based on the ideal body weight (BW) and using the value 880kJxBW^0.75 from FEDIAF[5] (3738 kJ/day). A gradual increase energy intake was applied over three days, 0.15-0.3 DER. A commercial diet suitable for puppies was given. Although the daily recommended amount of food was divided into seven meals and a maximum of 10% of the stomach volume (50mLxBW) was fed, the patient started presenting reflux when receiving 0.3 DER. Due to that, in the 7th day of hospitalization a central venous catheter was placed and PN was started. The calculated energy amount was slowly increased, from 0.2 to 0.3 DER in the first 8 to 16 hours, respectively. After 16 hours, 0.6 DER was achieved. The PN composition consisted in 58% of fat, 17% of dextrose, 25% of protein, 20mEq/L of K and 5mmol/L of P, reaching 657mOsm/L. Blood glucose, K and P were analyzed every 4 hours, triglycerides every 8 hours and the albumin, daily. After one day of PN, the albumin increased from 18g/L to 20g/L (ref 21-36g/L), while the P decreased from 1.81 mmol/L to 1.64 mmol/L (ref 1.65-3.36 mmol/L). In the 9-10th day of the dog's hospitalization, EN (0.1-0.15 DER) was combined with the PN (0.5-0.45 DER), but the dog continued presenting reflux. Consequently, EN was discontinued. Due to the previous decrease in the blood P, the P concentration of the PN was increased (10 mmol/L). Subsequently, the P value normalized (1.75 mmol/L). Three days after the PN, EN through the gastric tube was gradually introduced (until 0.6 DER) and in the 16th day of hospitalization the animal started eating orally and was discharged.

Conclusion. The EN is normally preferred over PN due to its benefits in the gut health. Gastric tubes are usually recommended considering the reduced risk of gastroesophageal reflux and aspiration compared to other EN routes in tetanus patients[6]. Despite the lower risk, some patients may still present reflux, which increases the risk of aspiration pneumonia. This condition limits the use of EN and, in these cases, PN should be considered.

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Nutritional management of muscle atrophy in a horse: a Case Report

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Introduction. The loss of muscle mass is called atrophy [1]. It can be due to myogenic damage or resulting from neurologic damage or atrophy. The myogenic generalized muscle atrophy can be induced by several causes such as: decrease of food intake, malnutrition, malabsorption or chronic diseases like pituitary pars intermedia dysfunction.[2] Although, a muscle condition score system is commonly used for dogs and cats, in horses such a system has not been validated yet.[3] Nevertheless, evaluation of equine muscle condition is required during physical examination.

Case history. An 8-year-old gelding Ardennes draft horse, 640 kg and body condition score (BCS) 2/9 with moderated to severe muscle atrophy, was presented at the service of internal medicine of Ghent University due to extreme weakness. After clinical examination, including blood analysis, teeth examination, and a glucose absorption tolerance test, the only potentially associated abnormality was low blood vitamin E (1,9mg/l). The horse was therefore referred for full nutritional assessment. The current diet was given for 5 month and was based on 18 kg of haylage (Pre -dried Italian rye grass, dry matter 55-70%) and 2.4kg of a fortified commercial feed was fed (Aveve condition mix®). No additional vitamin/mineral supplementation was added to the diet. The horse did not have access to the pasture, only to a small bare paddock. The exercise consisted of walking 2 hours once a week. His weight loss evolved gradually. **Nutritional recommendation and Discussion.** The ideal body weight of the horse was estimated at 711kg by increasing his current body weight by 5% per point of BCS. This ideal body weight was calculated with a BCS of 4/9.[4] A weight gain program was started not only to increase his body weight but to recover his muscle mass. This animal was not considered to require a “refeeding programme”. The new diet consisted of good quality hay (min 12 kg); Aveve all in soup®, 600gr; alfalfa pellets, 1,5kg; corn oil, 100 ml; and Cavalor Nutri Plus® 80gr. These adaptations allowed us to increase 40% the energy, and almost doubled (97%) the protein compared to his energy and protein requirements. Special attention was paid to vitamin E, not only to cover the maintenance requirements or the previous deficiency detected, but for the addition of oil to the diet (1IU/KG of body weight + 1IU/ml of oil). A neutral salt lick stone was provided and a gradual increase in physical exercise to help build up muscle mass was also recommended.[2] **Conclusion**. The horse went home after one week of hospitalization. For his clinical signs, an individually tailored feeding regimen was the only change for this horse to recover his normal condition. Only 5 weeks after starting the new diet, the horse gained 123kg and partially recover muscle mass. At home, the weight was estimated by taking some measurement of the horse and by using the following formula: [Chest circumference² (cm^2) x length (cm)]/11.877 = body weight (kg).[4] A new feeding advice was provided in order to help increasing muscle mass but stop weight gain.

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Diabetes mellitus and adverse food reaction in a dog – case report

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Introduction: Therapy of a dog with diabetes mellitus (DM) includes insulin therapy as well as dietetic management [1]. In patients with DM and additional diseases (e.g. adverse food reactions, AFR) the nutritional management is more challenging [2].
Case History: One year old dog, male, castrated, body weight 11.1 kg (estimated ideal weight 16 kg), BCS 3/9; Symptoms of polyuria, polydipsia, polyphagia, weight loss, recurrent diarrhoea. Differential diagnoses were excluded, DM was diagnosed, an insulin therapy was started and a commercial diet to support the digestive tract (diet A) was recommended by the treating veterinarian prior to referring it to the Institute of Animal Nutrition of the University of Zurich (ITE).
Nutritional consultation (table1): Neither an increase of energy supply by increasing the amount (B) nor adding millet flakes (C) lead to body weight gain. A commercial diet with an increased energy density (E) and adding a homemade portion to this diet (F) made the dog gain weight but the control of the blood glucose (CBG) was poor. A homemade diet based on lamb meat and millet (G) lead to a better CBG. As the dog still suffered from recurrent diarrhoea, the referring veterinarian recommended a commercial single protein diet (H) to assess potential adverse food reactions (AFR). The diarrhoea improved but the CBG was impaired even if adapted with a homemade portion (I) which might be due to the high glycaemic index of carbohydrates from parsnip. With a homemade diet recommended by the ITE based on horsemeat, cottage cheese, millet, beetroots, guar gum, cellulose and a mineral-vitamin-supplement (J) the CBG was improved as long as the amount of millet flakes was limited, despite the NfE content of J exceeded the one of G.

Table 1 Body weight at the start of feeding the diet and nutrient composition of diets A-J

Diet	BW (kg)	ME (MJ)	CP (g)	CP % DM	SF % DM	CF % DM	Nfe % DM	Ca % R	P % R	Carbohydrate source
A	11.1	3.9	70	32.0	21.5	1.8	39.3	301	287	rice
B	11.1	5.4	97	32.1	21.5	1.8	39.4	417	397	rice
C	11.1	5.4	104	34.3	17.5	4.5	39.9	193	241	rice, millet
E	11.1	5.4	84	30.2	25.9	2.1	36.7	259	257	corn
F	12.8	5.4	88	32.1	28.8	4.2	28.8	170	191	corn, millet
G	13.0	5.4	101	36.2	27.6	5.6	23.3	160	157	millet
H	14.2	5.4	122	40.7	24.3	5.9	23.7	224	339	parsnip, millet
I	14.5	5.7	120	38.6	20.6	3.7	31.5	233	291	parsnip, millet
J	15.6	4.2	108	41.9	11.6	6.0	35.7	192	181	millet

Body weight (BW), metabolizable energy (ME), dry matter (DM), crude protein (CP), Soxhlet fat (SF), crude fiber (CF), Nitrogen free extracts (NfE), calcium (Ca), phosphorus (P), recommendation (NRC 2006; R)

Outcome and Discussion: The body weight and blood glucose remained in a stable range but did not normalize. Due to a lower glycaemic index quinoa instead of millet might be beneficial in this patient. A very good owner compliance and the strict feeding of the calculated homemade diet successfully supported a dog with DM and AFR.

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Clinical signs and serum protein level enhancements in 3 dogs with PLE solely treated with a home cooked diet

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Introduction: Protein-losing enteropathy (PLE) is a syndrome that involves the lymphatic system. It is characterized by severe loss of serum albumin across the gut and is often related to chronic enteritis. Due to hypoalbuminemia and the severity of clinical signs, PLE could be challenging to manage in dogs [1]. A low-fat diet is the most effective approach of PLE management in human medicine [2], and there is evidence that also dogs, especially younger animals, can completely respond to a dietary treatment based on fat restriction [3]. Home cooked diet (HCD) is useful to obtain a considerable fat restriction and to restore the patient's appetite, but often it is replaced by commercial low-fat diet after a few weeks [4]. The aim of this case study was to assess the effect of a 1-month dietary management in 3 PLE dogs, fed a complete standardized HCD, on clinical scoring systems (CIBDAI and CCECAI), serum albumin (ALB) and total protein (TP) levels.

Animals, materials and methods: Three dogs (2 female crossbreds, 11y, 11.4kg and 13.5kg BW, BCS 4/9; 1 Bolognese male, 10y, 3kg BW, BCS 3/9) diagnosed with PLE based only on clinical signs (due to inability to perform biopsies quickly, before starting any medical treatment), as ascites 1/3, vomiting 1/3, diarrhoea 3/3, weight loss 3/3, lethargy 2/3 and hypoalbuminemia (< 2.6 g/dL), without evidence of other protein-losing conditions, were fed a complete standardized high protein (CP 101 g/Mcal), low-fat (CF 14.5 g/Mcal) HCD [composition (% on wet basis): 56% potatoes, 26% turkey breast, 9% cod fillet, 7% zucchini, 1% sunflower oil, minerals/vitamins supplements] based on their daily MER. Treatment success was evaluated based on CIBDAI and CCECAI scores [5,6] along with ALB and TP levels at day 0 (time of diagnosis) and after 30 days of HCD feeding. Statistical evaluation was performed using Student's paired *t* test, significance levels were set at $p \leq 0.05$ and $0.05 < p < 0.1$, respectively.

Results and discussion: Acceptance of HCD was high in all dogs and no side effects occurred. Results are presented in Table1.

Table1. Data (mean ± sd for 3 dogs) collected at days 0 and 30

Variable	Reference range	d0	d30	*P*-value
CIBDAI	0 normal to =/>9 severe	7 ± 2	1.33 ± 1.53	0.02
CCECAI	0 normal to =/>12 severe	7 ± 2	1.33 ± 1.53	0.03
ALB	2.75-3.85 (g/dL)	1.33 ± 0.25	2.57 ± 0.29	0.02
TP	5.6-7.3 (g/dL)	3.24 ± 0.68	5.72 ± 1.27	0.08

CIBDAI, canine IBD activity index; CCECAI, canine chronic enteropathy clinical activity index; ALB, serum albumin; TP, serum total proteins.

In all dogs treated with HCD, ALB was increased after 30 days but stayed within the reference range in only one of them who had the highest level at diagnosis (1.41 g/dL at d0 vs. 2.9 g/dL at d30). TP showed a general tendency to increase (p=0.08). Both CIBDAI and CCECAI scores showed a clear improvement in all dogs (p=0.02 and 0.03, respectively).**Conclusion**: This case study shows that feeding complete standardized HCD can represent the single therapeutic choice, with the direct advantage of avoiding the concomitant use of additional drugs, not only for diet-responder young dogs but also for aged dogs, wherein the use of immunosuppressive agents may not be recommended.

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Session 2B Energy requirements; Fatty Acids

Chair: Pat Harris

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Evaluating maintenance energy requirement in adult cats: preliminary results

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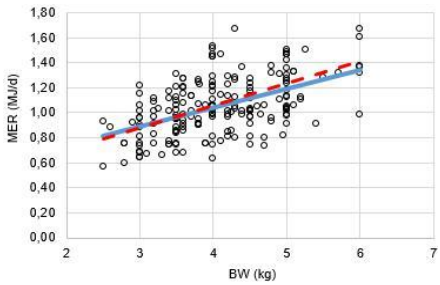
Introduction. Energy required to support energy equilibrium over a long period of time is defined as maintenance energy requirement (MER) [1]. The National Research Council (NRC), recommends the equation 0.42 MJ/kg $BW^{0.67}$ for maintenance in adult cats [2], while a recent meta-analysis by Bermingham *et al.*, proposed 0.24 MJ/kg $BW^{0.966}$ for light and normal cats (up to 5.5 kg BW) [3]. This study aims to determine an allometric regression equation for MER in adult cats, based on daily metabolisable energy [ME (MJ/day)] intake and body weight [BW (kg)]. At the same time, factors potentially affecting energy equilibrium will be explored. The same work has already been published for dogs [4].

Materials and methods. As part of an interactive teaching workshop, every year (2001-2019) students from the University of Toulouse (France), were tasked to follow an adult cat (≥ 7 months) for as long as 2 months. Details on 549 cats were collected. Cats could be either their own or belong to a close relative or friend. Data related to age (years), breed, gender, neutering status, BW (kg), pet food type, average food intake (g/day), body condition score (BCS) on a scale of 9, voluntary physical activity (calm, normal or active), lifestyle (indoor, indoor-outdoor or outdoor), disease (no or yes; if yes, which) and treatments. Pet food's ME was measured via NRC crude fibre formula [5]. Cats' weight had not to vary by more than 5% during the observation period, making for an unchanged BCS. Statistical work was performed via RStudio (R version 3.5.2).

Results and discussion. To develop our equation, only adult, neutered healthy Domestic Shorthair cats (DSH) with stable ideal BW (BCS 5/9) were considered, resulting in the allometric equation 0.46 MJ/kg $BW^{0.58}$ (adjusted R^2 0.33). Comparison with NRC allometric equation (0.42 MJ/kg $BW^{0.67}$) showed no statistical difference (p=0.18) (Figure 1), confirming findings by Thes *et al.* [6]. Looking into possible factors affecting MER (age, gender, neutering status, breed, overweight, activity, lifestyle, ownership, disease), only physical activity and lifestyle were found statistically significant. Active cats had higher energy requirements than calm cats (p=0.02), justifying a MER correction factor of 1.08. Observations on lifestyle showed that cats living outdoor (p<0.001) and indoor-outdoor (p=0.001) had higher requirements compared to indoor cats, accounting for a MER correction factor of 1.20 and 1.09, respectively. Considering the diversified ownership of cats in the study, and the fact that students at the University of Toulouse come from all across France, data collected can likely be extrapolated to the feline population of France.

Figure 1. Predicted requirements from the present study (—) and those of NRC (- - -).



Conclusion. The found allometric equation is close to the NRC (2006) recommended allowance for maintenance of lean adult cats. Use of a MER correction factor is recommended for active cats (k=1.08) and for cats living outdoor (k=1.20) or passing an equal amount of time indoor and outdoor (k=1.09).

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Old and adult dogs differ in body composition, energy expenditure and insulin secretion

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Introduction. Metabolic alterations in aging dogs might include lower insulin sensitivity and reduced lean mass (LM) with proportional increase on fatty mass (FM) resulting in sarcopenia. The comprehension of this metabolic alterations might help to develop tools to minimize them, potentially helping to favor a health aging process. The aim of this study was to compare old versus adult dogs regarding body composition, nutrient digestibility, nitrogen balance, energy intake to maintain constant body weight (EI_{BW}), protein turn-over through renal excretion of 3-methil-histidine (3-MTH), and postprandial glucose and insulin responses.

Animals, material and methods. Ten adult (2.2±1.0 years) and 10 old (12.9±1.6 years) beagle dogs were used, distributed in 4 Latin squares with 5 dogs each (approval 004824/17). Five moderate protein diets (16.3±0.3% of CP) with similar chemical composition, differing only in protein sources were used. After 12d of adaptation, total collection of faeces and urine were conducted for 7d to determine nutrient digestibility, nitrogen balance and creatinine renal excretion (CRE) and 3-MTH. Blood kinetics were performed (days 20 to 23) to measure postprandial glucose and insulin. On day 28, blood samples were collected before and after 2h of deuterium subcutaneous injection to measure body composition [1]. EI_{BW} was calculated based on 28d of food intake and weekly body weight measures. Data were submitted to variance analysis in a Latin square design, with treatments organized in a factorial arrangement (2 ages x 5 diets). There was no diet effect or diet x age interactions ($P>0.05$), so the age effects verified were presented ($P<0.05$). **Results and discussion.** Nutrient digestibility, nitrogen balance, urine production and 3-MTH renal excretion were similar between age groups ($P>0.05$). The LM content was lower (73.1±1.5% vs 78.1±1.5%; $P=0.013$) and the FM higher (26.9±1.5 vs 21.9±1.5 %; $P=0.013$) for old animals. CRE was lower in old dogs (20.7±3.8 vs 26.9±5.7 mg/kg BW/d; $P<.001$), this may be explained by the sarcopenia process [2,3]. EI_{BW} tended to be higher in adult versus old dogs (132±5.0 vs 120±5.0 kcal/kg$BW^{0.75}$/d; $P=0.052$), as previously observed [4]. Basal glucose was higher for adult dogs (82.5±5.0 vs 79.5±5.3 mg/dL; $P=0.002$). A tendency of higher maximum glucose (104±2.13 vs 98.6±2.12 mg/dL; $P=0.054$) and glucose area under the curve (AUC; 833±16.8 vs 800±16.7 mg/dL/h; $P=0.079$) were verified for old dogs. Basal (51.7±3.7 vs 40.2±2.3 pmol/L), mean (209±14.9 vs 147±14.0 pmol/L), maximal (427±125 vs 331±125 pmol/L), and insulin AUC (2440±266 vs 1548±252 pmol/L/h) were higher for old dogs ($P<0.01$), characterizing lower insulin sensitivity.

Conclusion. Older dogs require less EI_{BW}, have lower LM and require higher insulin secretion to control blood glucose in comparison to adult dogs.

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Elderly obese cats require lower energy and higher protein intake to maintain the rate of weight loss

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Introduction. Obesity in felines has a high prevalence and may result in many deleterious health effects [1]. This condition is frequently observed in elder cats as a consequence of lifespan increase. Considering this, it is important to manage obesity with an adequate weight loss program (WLP) associated with an adequate diet [2], especially when considering animals in a sarcopenic process, such as elder cats. This study aimed to evaluate a WLP adequacy and a diet profile efficiency for aging cats.

Animals, material and methods. The first group consisted of 10 cats with mean age of 12.00±1.10 years, body condition score (BCS) [1] of 8.00±0.81, fat mass of 37.18±8.24%, and lean mass of 62.81±8.24%. The second group was composed of the same obese cats after a controlled loss of 20.00% of their initial body weight (BW) (means: BCS: 5.70±0.82; fat mass: 30.27±9.90%; and lean mass: 69.72±9.90%). Cats were fed a high-protein (133g/1000kcal), low fat (31.6g/1000kcal) and low-starch diet (50.6g/1000kcal) [metabolizable energy (ME) 3.16 kcal/g as fed]. Protein and total energy intake were measured daily. BW was registered weekly and energy intake [ME/(kg body weight)$^{0.4}$] adjustments were made to keep weight loss rate around 1.00% per week. The WLP duration was 164.5±32.25 days.

Results and discussion. Fat mass decreased from 39.70% (27.20-48.00) to 32.30% (15.60-41.90) but the energy consumption for weight loss was smaller than the expected [2] to maintain weight loss rate around 1.00% (Figure 1), which is recommended by [3] to preserve lean body mass. This effect may have occurred due to muscle loss, since energy expenditure is directly associated with body muscle mass (MM) [4]. MM reduced from 3.18kg (2.47-4.13) to 2.81kg (1.96-3.96) and may indicate that protein intake was not sufficient during WLP or that a high amount of BW was lost [2]. Protein requirement is higher during WLP than in maintenance [5] and despite the 3.10g protein/kg/day intake is considered sufficient to adult cats [6], its requirement may be higher for elder cats and it was not sufficient trough all WLP (Figure 2), considering the progressive increase on energy restriction imposed to maintain weight loss rate.

Figure 1. Energy intake (EI) estimated in comparison to the EI required for mature cats fed a 133g CP/Mcal diet to maintain weight loss rate at 1.00% per week.

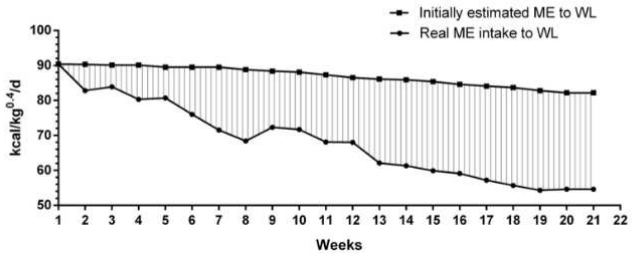
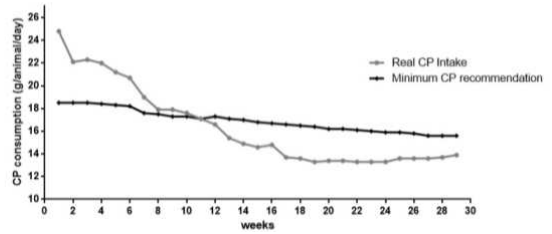


Figure 2. Mean of weekly crude protein (CP) requirement according to Fediaf (2019) and real CP intake of mature cats fed a 133g CP/Mcal diet to maintain weight loss rate at 1.00% per week.



Conclusion. Mature cats require less energy to maintain a weekly weight loss rate equivalent to 1.00% and a diet containing 133g/1000kcal protein was not sufficient to prevent muscle mass loss during WLP. A higher level of protein inclusion in weight loss diet and a less intense energy restriction are recommended for mature cats.

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Metabolizable energy prediction equations proposal for wet diets for dogs

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Introduction. Considering the evolution of wet food for pets, especially after the development of traditional energy prediction equations [1-3]; the use of new ingredients with greater digestibility; the increase in product diversity, the importance it has in the daily practice of animal nutrition; as well as the need to evaluate the applicability of predictive equations in different regions of the world. New equations to predict the metabolizable energy for these components are necessary, which is the aim of this work.

Animals, material and methods. A total of 49 products for dogs sold in Brazil were evaluated. The metabolizable energy of the products' labels was only considered for inclusion in the analyzes of the present study when acquired from companies that did in vivo evaluation, using the total feces collection method (with or without urine collection) or using indicators [1], and not through predictive equations. Bromatological information was also acquired with the respective companies. For the assumption of equations more applicable to wet foods, regression analysis was performed in SAS, with the aid of the PROC REG function for linear and multiple equations.

Results and discussion. Two assumptions were made (new proposed equation I and II), the main difference between the equations was the inclusion of non-nitrogenous extracts (NNE) as a quadratic regression coefficient in equation II versus only 1st degree linear in equation I and consequently, it improves its fit (Figure 1).The proposed equations that are most applicable to wet foods are as follows:

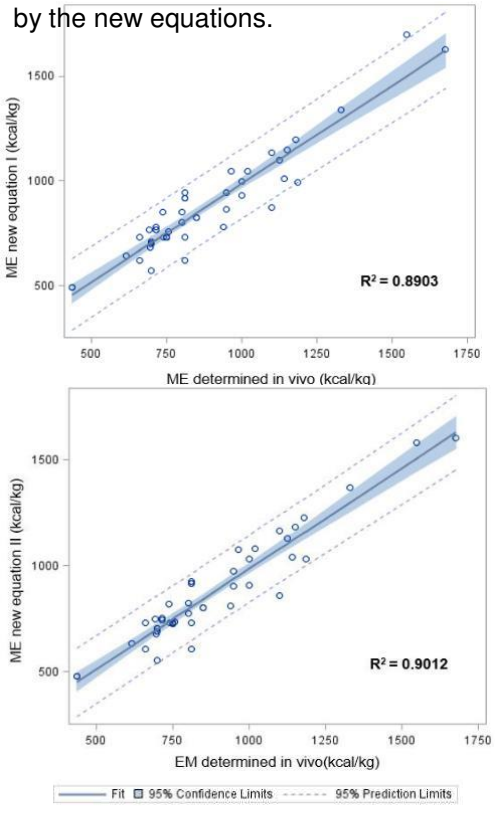
New proposed equation I (R^2 = 0.8903): ME (kcal / kg) = (4.5 x g crude protein / kg) + (9.3 x g ether extract / kg) + (4.5 x g NNE / kg);

New proposed equation II (R^2 = 0.9012): ME (kcal / kg) = (4.2 x g crude protein / kg) + (9.4 x g ether extract / kg) + [(5.8 x g NNE / kg) - (0.0080 x g NNE / kg ^{2})].

The coefficient of determination, also called R^2, is a measure of adjustment of a statistical model, to the observed values of a variable. The values of R^2 obtained in the new populated equations, were then compared to the traditional equations Atwater [1] (R^2=0.88) Modified Atwater [2] (R^2=0.88) and NRC (2006) [3] (R^2=0.85), being superior to all of these.

Conclusion. An assumption of applicable equations for the estimation of the metabolizable energy of wet foods was developed, contributing to the daily practical application of the professional working in the area of dog nutrition.

Figure 1. Regression analysis that illustrates the relationship between the metabolizable energy content (ME) in wet foods (n = 49) determined by means of animal tests and estimated by the new equations.



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Effect of dietary EPA and DHA supplementation during last month of gestation on plasma fatty acids composition of Charolais calves and cows

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Introduction. Fatty acids (FA) are of utmost importance in the perinatal period for the development of the central nervous and immune system [1]. The transport of polyunsaturated fatty acids (PUFA) through the placenta is considered to be minimal in ruminants, nevertheless, the FA of the cow are the main significant source for the calf during gestation [2]. Even though, n-3 FA milk enrichment of dairy cows is widely researched, there is limited data on the adaptation of n-3 long chain PUFA (LC PUFA) metabolism during gestation in cows [1,3]. Therefore, the aim of this research was to investigate the influence of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) supplementation during the late gestation on FA metabolism of cows and to determine whether it would affect FA composition of newborn calf plasma.

Animals, materials and methods. In the feeding trial, a total of 20 Charolais cows during the last month of their gestation were included. All the cows were fed a basal TMR diet, consisting of haylage and corn concentrate. The cows were divided into a control group (CON) and an experimental group (EPA+DHA). The latter received a fat supplement (FATMIX 65, AND Nutrition, Spain) at the amount of 100 g/day (9.1 and 7.8 g/cow/day of EPA and DHA, respectively). Blood samples were collected on the day of calving from cows and their calves (*v. jugularis*, plasma). Analysis of FA composition was performed by Shimadzu GC2010Plus Gas Chromatograph, equipped with a flame ionization detector and a 30-m capillary column ZB WAX (Phenomenex, Torrance, CA, USA). Data were analyzed using GraphPadPrism6 software between the groups of cows and calves using the unpaired t-test ($p<0,05$) (GraphPad Software, Inc., San Diego, CA, USA). This study was approved by the institutional ethical committee. **Results and discussion.** In cow plasma, concentrations of PUFA and n-3 PUFA were significantly higher in the EPA+DHA group ($p<0,05$). FA composition of the plasma in EPA+DHA group was significantly altered: significantly higher concentrations were determined for alpha-linolenic acid, linoleic acid, EPA ($p<0,05$), arachidonic acid ($p<0,001$) and docosapentaenoic acid ($p<0,01$). Meanwhile, the plasma FA composition of calves was not affected by the supplementation of dams during gestation ($p>0,05$). **Conclusion.** Supplementation of low dosage EPA and DHA, during gestation, alters plasma composition in cows and enables a pool for incorporation in milk and peripheral tissues. The observed sparing effect of essential fatty acids: ALA and LA, as well for other LC PUFAs is visible in the EPA+DHA group of cows. Meanwhile, the same effect was not detected in calf plasma which suggests a limited transplacental transfer of LCPUFA or fast utilization during the last month of gestation [2,4].

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Lipid and glycemic response of diabetic dogs fed omega-3 fatty acids

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Introduction. Previous studies with diabetic human suggested that fish oil reduced lipemia and there are controversies about its effect on glucose concentration [1]. It is controversy for healthy dogs [2]. We believed Ω-3 could reduce lipemia and glycemia of diabetic dogs. It is the first study focused on Ω-3 metabolic effect for diabetic dogs. **Animals, material and methods.** The Ethics Committee approved this study. Eleven stable diabetic dogs (no other diseases, body condition score 4-6/9[3], using insulin, no drugs or treats), were submitted to two 12-week dietary periods. Dogs stayed with their owners and received (cross over, double blind and randomized) two complete (FEDIAF) high-fiber diets: Control - CO (dry matter basis: 16.3% fat; 3.8% Ω-6; 0.5% Ω-3; 0.1% EPA+DHA) and Test - TE (5% fish oil inclusion; 18.3%; 3.6%; 1.6%; 0.9%).

A 9-day continuous interstitial glucose curve (Freestyle Libre®), fasting plasmatic lipoprotein (fast protein liquid chromatography), and a 10-hour plasma triglycerides (Trig) and cholesterol (Chol) curve (6-points, every 2 hours) were performed, with first point before first meal and first insulin. Wilcoxon or paired T test were used to test for significant differences between CO and TE ($p<0.05$).

Results and discussion. Dogs received 8.5±4.1mg EPA+DHA/kg body weight with CO, and 142.9±34.2mg/kg with TE. Higher Ω-3 resulted in lower mean (TE: 234.2±52.0 x CO: 278.8±48.6mg/dL; $p=0.01$), minimal (219.5±50.5 x 260.6±47.0mg/dL; $p=0.02$), maximum (249.3±55.7 x 301.8±54.4mg/dL; $p=0.01$), fasting (308.3±162.8 x 350.6±135.6mg/dL; $p=0.03$), and fluctuation of Chol (249.3±55.7 x 301.8±54.4mg/dL; $p=0.03$). There were lower levels of Chol in the curve (Figure 1A), lower levels of the Chol fraction in VLDL ($p<0.01$; Figure 1B), and in non-HDL lipoproteins ($p=0.05$). The serum triglyceride levels did not differ significantly between groups ($p>0.05$). After feeding the TE, there was greater interstitial concentration of mean total glucose (308.4±64.9 x 276.2±64.6mg/dL; $p=0.04$), nocturnal mean (312.8±69.7 x 277.5±71.8mg/dL; $p=0.02$), maximum in daytime (411.5±48,8 x 371.7±47.7mg/dL; $p=0.01$), and in nighttime (415.0±58.9 x 373.7±67.9mg/dL; $p<0.01$), longer time in hyperglycemia (53.1 x 40.4%; $p<0.01$), and less time in hypoglycemia (1.3x4.7%; $p<0.01$). Current study used the highest omega-3 dosage to perform metabolic evaluation of dogs.

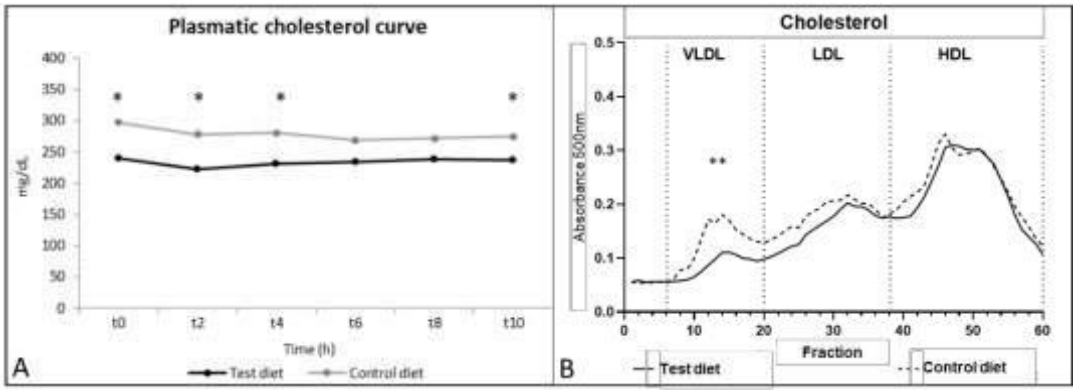

Figure 1 – Mean plasmatic cholesterol (A) and lipoprotein fraction (B) of diabetic dogs after intake of a control diet (CO, without fish oil) and a test diet (TE, 5.0% fish oil). Significant differences tested by *paired T test or **Wilcoxon test ($p<0.05$).

Conclusion. Fish oil reduced cholesterol and worsens glycemia in diabetic dogs.

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GO TO ABSTRACT LIST

Metabolizable energy in commercial extruded diets for dogs and cats: comparison of different predictive equations

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Introduction. In pet food, the energy value of the food is expressed in terms of metabolizable energy (ME), and the precise determination of the ME content of the food requires in vivo evaluation [1]. However, it is limited to research conditions and has a high cost; consequently, different indirect methods have been proposed over the years to estimate this content. Due to the different methods available, the evolution of the products marketed for dogs and cats, the use of new ingredients, increased digestibility of the products used, it is necessary to analyze the equations available in the literature and the reliability of their prediction in these circumstances, which is the objective of this study.

Animals, material and methods. A total of 338 products sold in Brazil were evaluated, of which 112 were extruded cat foods and 226 were extruded dog foods. The metabolizable energy of the products' labels was only considered for inclusion in the analyzes of the present study when acquired from companies that carried out in vivo tests, using the total feces collection method (with or without urine collection) or using indicators, and not through estimation equations. In relation to the determinations of metabolizable energy of foods, the prediction equations of Atwater (1902) [2], AAFCO (1997) [3], NRC (2006) [1] and FEDIAF (2019) [4] were used. Statistical analysis was performed in SAS program, for the comparison between the reference method (in vivo evaluation) and the other alternative methods, and ANOVA and orthogonal contrasts tests were performed ($p<0.05$).

Results and discussion. In all estimated equations, there was a non-conformity of the forecast to the evaluated reference method (animal testing) (Table 1), however, the Atwater factors (1902) for dogs and cats and the FEDIAF equation (2019) for dogs were the methods that showed less difference.

Table 1. Estimation and difference between methodologies for ME determination of dry extruded food for cats (n=112) and for dogs (n=226) sold in Brazil (mean ± standard deviation).

Variables	In vivo evaluation (reference)	Prediction Equations				P[1]			
		Atwater (1902)	AAFCO (1997)	NRC (2006)	FEDIAF (2019)	C1	C2	C3	C4
Extruded cat food									
Metabolizable energy (Kcal/Kg)	3,968.85 ± 232.96	3,824.82 ± 228.98	3,434.89 ± 223.24	3,647.57 ± 211.89	3,745.29 ± 210.89	<0.001	<0.001	<0.001	<0.001
Diferrence[2] (%)	-	3.53 ± 2.49	13.40 ± 2.36	8.00 ± 2.07	5.28 ± 2.47	-	-	-	-
Extruded dog food									
Metabolizable energy (Kcal/Kg)	3,726.25 ± 235.07	3,788.23 ± 178.48	3,396.77 ± 172.28	3,535.03 ± 189.67	3,788.23 ± 178.48	0.008	<0.001	<0.001	0.008
Diferrence[2] (%)	-	-1.92 ± 3.98	8.64 ± 3.50	4.96 ± 3.97	-1.92 ± 3.98	-	-	-	-

[1]C1 – In vivo evaluation (reference) x Atwater (1902); C2 – In vivo evaluation (reference) x AAFCO (1997); C3 – In vivo evaluation (reference) x NRC (2006); C4 – In vivo evaluation (reference) x FEDIAF (2019); [2]Difference of the result of ME obtained through animal testing compared to ME through the proposed prediction equation.

Conclusion. Based on the results of the present study, the oldest equation tested here seems to be the best predictor of metabolizable energy in nutritional practice.

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The cognitive function of growing dogs improves with the supplementation of polyunsaturated fatty acid DHA

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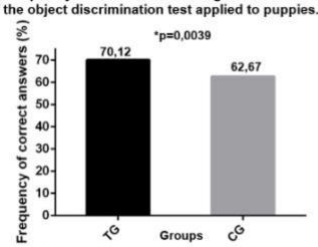
Introduction. It is highlighted in the literature that omega-3 polyunsaturated fatty acid, [docosahexaenoic (DHA)] plays an important role in the development of cognitive function [1,2]. However, the objective of this study is to evaluate the hypothesis that with a concentration of DHA higher than those tested in the literature through capsules [1,2] it is possible to promote greater learning and health benefits for growing dogs.

Animals, material and methods. 12 healthy dogs (three months old) were used, blocked by breed and randomly distributed in two groups (n = 6): Control Group (CG), without supplementation and Test Group (TG), with supplementation of 40mg DHA/kg/day. The data for the variables: object discrimination test adapted from [3] [to measure learning ability associated with cognitive function, consisting of a normal (NP) and a reverse (NR) phase], serum PUFA concentration by gas chromatography coupled to mass spectrometry [DHA + Eicosapentaenoico (EPA), arachidonic (AAA), Linoleic (AL), alpha linolenic (AAL) acids] and total antioxidant capacity (CAT) according to [4] were collected before (0), 30 (1), 60 (2) and 90 (3) days after the start of supplementation. The results obtained were analyzed in the SAS statistical program, the Shapiro Wilk test was used to verify the normality of the residues and the Tukey test for comparison of means ($p<0.05$).

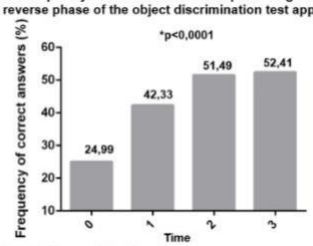
Results and discussion. The results obtained in the NP showed differences between treatment ($p=0.0039$) and time ($p<0.0001$). The Frequency of correct answers in the TG (70.12%) was higher than for CG (62.67%) (Figure 1). In relation to the RP, there was only a time effect ($p<0.0001$) (Figure 2). The animals in the TG group obtained greater learning capacity during the NP regardless of time, while in the RP, there was an increase in the correct responses, regardless of the treatment. When obtaining difficulty when compared to the CG group during the ER. In addition, the degree of repetition of the test may have influenced the increase in correct responses in this phase, as found by [4]. The concentrations of EPA + DHA at time 1, 2 and 3 were higher than at time 0 for the TG group ($p=0.0159$), which in turn showed a higher concentration at time 2, when compared to the CG ($p=0.0245$).

Figure 1. Frequency of correct answers regardless of time in the normal phase of the object discrimination test applied to puppies.



CG = Control Group; TG = Test Group

Figure 2. Frequency of correct answers for period regardless of treatment in the reverse phase of the object discrimination test applied to puppies.



CG = Control Group; TG = Test Group

AAA concentrations increased over time, regardless of treatment ($p=0.0158$), as well as AL ($p<0.0001$), while for AAL there were no differences ($p>0.05$). The mean serum percentages of EPA + DHA were 1.84% + 3.04% and 2.04% + 3.56%, for TG and CG respectively. For CAT, there was a time effect ($p=0.0322$) regardless of treatment, which demonstrates that higher concentrations of polyunsaturated fatty acids did not interfere with serum oxidation as found by [5] LeBlanc et al. (2005).

Conclusion. It is possible to conclude that the cognitive function of growing dogs can be improved by providing fish oil concentrated in docosahexaenoic polyunsaturated fatty acid (DHA) without increasing serum oxidation.

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Use of an *in vitro* gas production technique to evaluate horse diet containing linseed oil

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Introduction: Intensive training of sport horses leads to increased energy demands. The most common way of meeting their high energy requirements is to enrich diet with concentrate feed, which in turn may cause metabolic disorders, due to high content of fast-fermenting starch [1]. For this reason, various types of oil may be used as an alternative energy source [2]. However, large doses of oil in the diet have been reported to negatively affect the digestibility of fibre fractions in the gastrointestinal tract [3]. Therefore, the aim of this study was to determine the effect of different level of dietary linseed oil supplementation in non-working horses which faeces were used as an inoculum source on the *in vitro* gas production parameters of chosen feeds.

Animals, material and methods: Study was carried out on 6 horses (Konik Polski mares, 3 - 12 years old) with a BW of 200 - 370 kg. Horses were individually housed in box stalls with free access to water and mineral lick. Daily feed rations were calculated individually basing on NRC (2007) guidelines for non-working horses. A 3 x 3 repeated Latin square design was used, with 3 groups of animals and 3 doses of linseed oil (0 (control group), 150 or 300 ml of oil per day per horse, which corresponds to 0-1.5 ml/kg BW). Each horses received meadow hay (80% of DM diet) and concentrate. The study consisted of 3 consecutive periods, each lasting 24 days (14 days of adaptation and 10 days of data collection period; 72 days in total). Daily dose of oil was administered with concentrate feed twice a day, during morning and evening feeding (at 7 am and 5 pm). In the last day of each period, fecal samples were collected rectally and transported immediately to the laboratory where were used as an inoculum source. The gas production was tested using Menke & Steingas technique [4] with 50-ml glass syringes and grinded (0.5 mm) samples of meadow hay and oat grain. Readings were taken at 2, 4, 6, 24 and 48 h of incubation and served for calculation of cumulative net gas production (ml/g DM). Effect of the diet on in vitro gas production in groups defined by level of oil was analyzed using ANOVA and Kruskal-Wallis test in R software.

Results and discussion: Total net gas production was not affected by the amount of oil introduced to the diet of horses [Table 1]. Also, the gas production rate was not different between treatments for each of tested feeds.

Table1. Effect of dietary linseed oil on the cumulative gas production and IVOMD[1] Treatment (mg/g DM)

Feed	Control oil 0 ml	Oil 150 ml	Oil 300 ml	p-value
Meadow hay	20.38 (33.24)	18.76 (32.79)	14.40 (29.53)	0.68
Oat grain	36.35 (52.38)	38.46 (55.94)	34.69 (51.79)	0.14
Blank	4.00 (17.55)	3.17 (17.25)	3.00 (16.81)	0.58

[1]IVOMD- *in vitro* organic matter digestibility in brackets (%)

Conclusion: Linseed oil supplementation, even in large doses, does not significantly affect fermentation processes in the gastrointestinal tract of Konik Polski horses and probably has no effect on the composition and activity of microbiota. However, this is preliminary study and further research should cover a larger group of horses and also other breeds. The study was supported by the Ministry of Science and Higher Education in Poland (DS-3217/KZiDZ/2018).

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Session 3A Fibre

Chair: Ana Lourenço

Effects of increasing dietary rye levels on viscosity and DM-content of digesta as well as on stomach emptying in young pigs

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Introduction: The high levels of arabinoxylans and fructans of rye might have an impact on physical properties of the digesta, e.g. on its viscosity and DM-content in the anterior gastro intestinal tract of pigs [1]. The experimental studies, mentioned below, aimed on a characterization of young pigs' digesta regarding viscosity and DM-content. Moreover, it was of interest to determine its impact on digesta passage through the stomach.

Animals, materials and methods: Forty pigs (age: 46.8 ± 5.28 days; bw: 16.1 ± 4.13 kg) were housed individually in four groups of 10 pigs each. Each group was fed a diet consisting of wheat and/or rye, barley, soy, potato protein and a mineral supplement. The sum of wheat and rye was 69 % in all diets, whereby the compound feed of each group was characterized by a different ratio (%) of wheat to rye (wheat/rye: 69/0; 46/23; 23/46; 0/69). After 4 weeks of different dietary treatments, the pigs were dissected. Before this, the diets were offered again about 4 hours *ad libitum* (after 12 hours feed withheld). In dissection mass and DM-content of stomachs' digesta were detected. Moreover, extract viscosity in digesta along GIT were determined. Statistics were done by SAS® ($p < 0.05$; Anova).

Results and discussion: With increasing dietary rye levels DM-content in stomachs' digesta rose significantly (group 1: 197^{a} ± 49.2; group 2: 236 ± 108^{ab}; group 3: 265 ± 81.8^{ab}; group 4: 353 ± 66.1^{b}) and the extract viscosity increased in feed, gastric digesta and the content of small intestine (Tab. 1). Moreover, compared to group 1 (69 % wheat), stomach emptying in groups 2-4 was slightly lowered (~13 %↓).

Tab. 1: Stomach emptying (g DM/h) and extract viscosity (mPa*s) in feeds and digesta of young pigs

group	stomach emptying	extract viscosity				
		feed	stomach	small intestine	caecum	colon
1	80.9 ± 17.3	1.47 ± 0.137^{a}	2.03 ± 0.63^{a}	2.33 ± 0.959^{a}	2.32 ± 0.429	12.2 ± 8.90
2	68.7 ± 16.2	1.81 ± 0.216^{a}	8.14 ± 6.62^{ab}	3.23 ± 1.65^{ab}	2.15 ± 0.433	12.1 ± 9.45
3	70.0 ± 12.0	1.83 ± 0.358^{a}	16.8 ± 15.6^{b}	4.06 ± 2.78^{ab}	2.35 ± 0.285	11.7 ± 5.11
4	70.1 ± 20.7	2.78 ± 0.659^{b}	51.8 ± 12.5^{c}	6.45 ± 4.75^{b}	2.61 ± 1.09	7.7 ± 5.56

Conclusion: The marked differences in the diet as well as stomachs' and small intestines' content disappeared in caecum and colon, which means that most of viscosity-affecting constituents seem to be degraded mostly when reaching/enterig the caecum. With higher DM-content and extract viscosity the stomach emptying tended to be retarded in rye-groups - leading to a longer lasting stomach fill. Moreover higher extract viscosity of small intestines digesta could result in a slower glucose-absorption and a more moderate insulin response [2]. Due to the fact, that probably no postprandial hypoglycemia occurs this could result in a longer lasting satiety [3]. Thus, there could be advantages including rye in compound feeds when a longer lasting satiety is intended, e. g. in feeding of pregnant sows (regularly restrictive).

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Cortisol levels in saliva and colostrum in gestating and lactating sows fed different fibre sources

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Introduction. Parturition commonly involves stress for sows, which may be displayed as increased stereotypical behaviour and excretion of cortisol. A correlation between stereotypical behaviour, salivary and faecal cortisol levels was reported with a reductive impact of dietary fibre contents [1]. The aim of this study was to investigate the impact of two different fibre sources on salivary and colostrum cortisol levels and performance in sows during farrowing period.

Animals, materials and methods. Twenty sows (6 pluriparous, 14 gilts) were randomly allocated to two different trial groups receiving gestation (11,4 MJ ME/kg DM) and lactation (13 MJ ME/kg DM) diets containing either 15 % sugar beet pulp and 3 % lignocellulose (T1) or 3 % sugar beet pulp and 15 % lignocellulose (T2). The diets were based on barley, wheat and soybean meal. In the gestation period, the animals were kept in groups and housed individually one week ante partum until weaning at day 28. The sows were fed restrictively during gestation with, 3,5 kg feed for gilts and 4 kg for pluriparous sows the week prior to parturition, while ad libitum feeding was provided during lactation. Feed intake was recorded daily for the individual sows. The sows' body weight was measured 7 days ante partum and at weaning while the piglets were weighted at birth and at 2, 7, 14, 21 and 28 days of age. At parturition, colostrum samples were obtained from the sows. Saliva was collected prior to rehousing, directly and 24 h after parturition and 7 and 28 days post-partum. Saliva samples were collected using Salivette Cortisol (Sarstedt AG & Co. KG, Germany) and a metal clamp. Cortisol was measured in the saliva and colostrum using a commercial Cortisol ELISA Kit (IBL International GmbH, Germany). Statistical analyses were performed with the software IBM SPSS (Version 22, USA) and statistical differences ($p<0.05$) and subgroups were determined using the non-parametric Kruskal-Wallis test and Mann-Whitney U test for cortisol and t-test and Mann-Whitney U test when evaluating performance data. The animal trial was approved by the Regional Office for Health and Social Affairs (LaGeSo Reg. G0112/19).

Results and discussion. There were no significant differences between treatments regarding litter size at birth (11.4 ± 4.0 and 11.3 ± 3.5), average piglet weight at birth (1.52 kg ± 0.27 and 1.62 kg ± 0.39) or at weaning (7.2 kg ± 1.6 and 7.6 kg ± 1.2), and average piglet weight gain (5.7 kg ± 1.5 and 6.0 kg ± 1.2). Additionally, no significant differences were observed in the sows' body weight at farrowing (217 kg ± 34 and 195 kg ± 42) and weaning (209 kg ± 23 and 198 kg ± 39) or health related issues for T1 and T2, respectively. Salivary cortisol levels increased around farrowing for both treatments followed by a continuous reduction until weaning, indicating that parturition is a stressful period for sows. Cortisol levels in colostrum (T1: 230.5 ng/mL ± 167.5; T2: 305.3 ng/mL ± 238.7) significantly exceeded simultaneously obtained salivary cortisol levels (T1: 97.9 ng/mL ± 56.7; T2: 111.4 ng/mL ± 82.9), with the highest cortisol concentrations observed in T2 vs. T1 groups.

Conclusion. Colostrum but not salivary cortisol levels differed significantly between sows fed the different fibre sources. This could have an influence on the suckling piglets' behaviour and health. However, this needs to be further investigated.

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Mango peels and apple pectin as feed compound for weaner piglets

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Introduction

Mango peels and apple pectin are by-products from the food industry. Their composition is extremely interesting because they have a number of biologically active ingredients. Polyphenolic compounds have been described in the skins of mangos, including several flavonoids, xanthones, phenolic acids and gallotannins. The polyphenolic components of mango peel include mangiferin, quercetin, rhamnetin, ellagic acid, kaempferol and their conjugates. Apple pectin is considered as fermentable carbohydrate source, which might increase short-chain fatty acids in the caecum and colon and have an impact on the intestinal microbiota. The objective of the study was to explore the effects of mango peels and apple pectin in weaned piglets.

Animals, material and methods

A feeding trial was conducted to analyse the impact of 1 or 2 % of apple pectin or mango peels compared to a control diet in weaned piglets. Performance was measured over 6 weeks in 10 replicates per group with 2 piglets each, intestinal parameters were analysed in a total of 5 piglets/treatment after 4 weeks. Measurements included the apparent ileal digestibility of nutrients with titanium oxide as indigestible marker, the apparent total tract nutrient digestibility and the analysis of different bacterial metabolites (short chain fatty acids, lactate, ammonia, biogenic amines, phenols and índoles). Defined groups of the intestinal microbiota were determined by real time PCR, the substrate utilization potential of the microbiota was measured based on faecal samples with BIOLOG MT2 plates. Statistical analysis of the data was performed using IBM SPSS Statistics 21 (SPSS Inc., Chicago, IL, USA) with a p value ≤ 0.05.

Results and discussion

The performance of the piglets was not different between the 5 experimental groups. The apparent precaecal digestibility of crude protein was similar between the groups ($p=0.248$), while crude fat showed the highest apparent ileal digestibility ($p = 0.024$) compared to the other experimental groups with 2 % of mango peel. The apparent total tract digestibility did not indicate any differences for crude protein ($p=0.775$) and crude fat ($p=0.271$). When comparing the effects of mango peel or apple pectin on the bacterial metabolites and composition, only numerical differences were found. The differences were more apparent in the precaecal sections, indicating intensive small intestinal fermentation. A reduced proteolytic activity of the microbiota of the colon was shown in the experimental groups with 2 % mango peel or apple pectin. Compared to the control group, the supplemented experimental groups showed a significantly higher substrate utilization of mango peels and apple pectin when tested in the BIOLOG system.

Conclusion

Within the scope of this study, only minor effects of the addition of mango peel or apple pectin could be determined in piglets after weaning. Mango peels and apple pectin might have an interesting effect on the fermentation activity of the intestinal microbiota, which would need to be explored in detail in further studies.

Diversity of the faecal microbiota of sows before and after farrowing in 20 sow farms in Germany

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Introduction. It is known that the composition of the microbiota in sows is not constant, but depends on genetic, dietary and environmental conditions. Furthermore, the late phase of farrowing drastically changes physiological conditions of the animal. This may lead to changes in the microbiota as well. The aim of the study was therefore to screen the microbiota of sows before and after birth in 20 German pig farms to investigate possible changes in the microbial composition under practical conditions.

Animals, materials and methods. Between January and November 2019, 400 faecal samples were collected from sows in 20 German pig farms. In each farm, samples of 10 sows were taken 14 days before and 7 days after farrowing. DNA was extracted with a commercial kit (QIAamp PowerFecal Pro DNA Kit, Qiagen, Hilden, Germany) and sequenced by Illumina MiSeq 16S DNA Sequencing. Bioinformatic analysis was performed using the pipeline DADA2 [1]. The mean relative abundances of detected bacteria before and after farrowing were computed and evaluated by the non-parametric Mann-Whitney U-Test.

Results and discussion. At the phylum level, 10 farms showed significant changes in the relative abundance of the dominant bacteria (>1%) after farrowing. On most farms, the phyla *Actinobacteria* and *Bacteroidetes* decreased, while the *Firmicutes* increased compared to before farrowing. At the order level, the *Erysipelotrichales* showed a significant increase after farrowing in 15 farms. The *Coriobacteriales* and *Selenomonadales* significantly reduced their relative abundance after farrowing in 13 farms. Dominant genera that significantly increased after farrowing were *Romboutsia* (11/20), *Clostridium sensu stricto 1* (10/20) and *Turicibacte*r (9/20). *Lactobacillus* and *Streptococcus*, with few exceptions, had lower relative abundances after farrowing than 14 days before. Other genera with a lower abundance (< 1 %) were also affected differently in individual farms (data not shown). Overall, the results show that a shift in relevance within bacterial communities occurred for many different bacteria. This may lead to an altered susceptibility to disease and responsiveness to interventions on problematic farms with a history of microbial pathogens. The driver of microbial changes on individual farms could be individual changes in particular environments and management systems as well as feed changes combined with the particular physiological condition before birth and the suckling period. An integration of the intestinal microbiome into farm specific health strategies could therefore be a promising approach.

Conclusion. The microbial composition of the fecal sow microbiota changes significantly between different times of production and between individual farms. In the future, it may be worthwhile for problematic farms to screen the microbial composition of sows before intervention. However, this would also require knowledge about farm husbandry, feeding and –management in order to integrate this data to predict which type of intervention gives the best chance of success.

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Effect of diet transition and duration on a high-grain ration on faecal pH and particle size distribution in dairy cows

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Introduction. Diet concentrate level affects ruminal pH and may contribute to the onset of subacute ruminal acidosis (SARA), which may in turn influence hindgut fermentation and digestion [1,2]. On-farm faecal pH measurement and sieving can be used to assess overall gut fermentation and nutrient utilization [3]. This study was conducted to evaluate the effect of diet transition and days on a high-grain ration on faecal pH and faecal particle size distribution in dairy cows.

Animals, material and methods. Nine ruminally cannulated Holstein cows (initial BW=922±86.3 kg) were used. The feeding trial lasted 40 days. Cows were fed a forage-only diet for one week, then all cows were transitioned to a 65% concentrate ration over one week. Faecal pH and particle size distribution were measured weekly. Measurements were conducted when diet included 0% concentrate (day 5), 40% concentrate (day 13), and 65% concentrate (days 20, 27, 34 and 40). In each sampling day, faecal samples were collected rectally at 0, 4, 8, and 12 h relative to feeding. Faecal pH was measured; then, each sample (200 g) was wet sieved with a set of three steel screens with pore sizes of 2, 1.18, and 0.5 mm by running a steady stream of tap water on the sample. Data were analyzed with SAS with sampling day as fixed affect and cow as random effect.

Results and discussion. Faecal pH decreased ($p < 0.01$) when cows were transitioned from forage-only to the 65% concentrate diet. Overall, the proportions of faecal particles retained on the 2 mm screen were greater ($p < 0.01$) towards the end of the high concentrate feeding. Whereas particles retained on 1.18 and 0.5 mm screens decreased ($p < 0.01$) throughout the experiment (Table 1). Variations in faecal pH and particle size distribution may reflect changes in gut fermentation and digestion [4]. Specifically, it could be speculated that there was an impairment in fiber digestion with high concentrate feeding, likely due to negative effect of low pH on fiber digesting bacteria [5].

Table 1. Effect of diet change and duration on a high-concentrate ration on faecal pH and particle size distribution.

	Measurement day and level of concentrate							
	5	13	20	27	34	40		
Item	0%	40%	65%	65%	65%	65%	SE*	p-value
Mean pH	7.30	6.63	6.14	6.38	6.35	6.25	0.05	<0.01
Particle size								
2 mm, %	9.5	9.2	9.4	11.8	13.7	13.0	0.95	<0.01
1.18 mm, %	9.5	8.2	7.3	5.9	6.1	5.6	0.31	<0.01
0.5 mm, %	35.6	25.3	19.4	18.1	18.6	15.7	0.68	<0.01
<0.5 mm, %	45.3	56.9	63.3	64.2	61.6	65.4	1.55	<0.01

*Largest standard error of the mean

Conclusion. Dietary transition affected faecal pH and particle size distribution. Measurement of faecal pH and particle size represents a practical tool to diagnose SARA as well as assess the overall effect of grain-rich diet on gut fermentation and nutrient utilization.

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Dietary preference of rabbits *(Oryctolagus cuniculus*) offered feed formulation varying in abrasive size in pelleted or extruded form

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Introduction. Continuously growing (hypselodont) cheek teeth and incisors allow rabbits to compensate for tooth wear with reactive growth during their entire life [1]. However, this regeneration of dental tissue may be limited in its capacity if faced with extremely abrasive diets. Selective feeding behaviour based on diet energy content has been well described in wild rabbits [2]. Here, we investigated whether rabbits additionally choose their diet based on its inherent abrasive and chewing potential.

Animal, material and methods. We offered groups of experimental rabbits (n=14) different isocaloric nutritionally complete diets in preference experiments for 7 days with different diets to choose from per stage. Diets varied in abrasive size and origin (fine silt 4µm, coarse silt 50µm, fine sand 130µm, coarse sand 233µm; rice hulls) as well as diet matrix (pelleted or extruded). In stage 1, the animals could choose between a control pellet or the same with added 8% fine sand; in stage 2, between a control pellet and the same with added 8% fine silt, coarse silt or fine sand; in stage 3, between a control pellet, the same with added (phytolith-rich) rice hulls, and the latter with added coarse sand; in stage 4, between a pelleted and an extruded form of the same recipe, where the extruded diet had a lower density (1600 vs. 2850 g/L). Food intake was analysed using a paired samples t-test for stage 1 and 4 and repeated measures ANOVA with Sidak post hoc for stage 2 and 3.

Results and discussion. The rabbits preferred the abrasive diets compared to controls (stage 1: $P<0.001$), but they liked silt abrasives better than the fine sand (stage [2]. $P=0.001$). In stage 3, they preferred both the phytolith-rich diet and the one with additional sand over the control ($P<0.001$); numerically, they ingested more of the diet with rice hulls and sand than the rice hull diet, but this was not significant. In stage 4, they preferred the extruded over the pelleted diet ($P<0.001$). The rabbits avoided the low-abrasive control diets, suggesting that some dietary-related wear is desired, possibly to maintain a consistent stimulation of the tooth's wear/growth mechanism. The coarse sand grains caused significant wear on the incisors as well as the cheek teeth [3], yet this diet was preferred, while the fine sand diet was avoided. The preference for the less energy dense extruded diet, meant longer feeding times and increased chewing activity.

Conclusion. When given the choice between non-abrasive and abrasive diets, rabbits prefer abrasive diets, and prefer extruded diets over pelleted ones. The results underline the relevance of stimulating chewing and tooth wear in this species.

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Effects of red and white sorghum varieties, ground in different particle sizes, on fecal microbiota of cats

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Introduction. Despite not being commonly used in cat foods, sorghum has ecological and productive advantages, and adequate nutritional value in comparison to other carbohydrate sources [1]. The aim of this study was to compare the effects on fecal microbiota of extruded cat foods based on red and white sorghum, with different particle sizes, to brown rice or corn as the cereal source.

Animals, material and methods. Four diets were formulated for cat maintenance [2] to have comparable nutrient content (28±1.2% starch, 32±0.9% crude protein, 17±0.9% fat, 3±0.3% crude fiber), with different carbohydrate sources: brown rice (BR), corn (C), red sorghum (RS) or white sorghum (WS). The BR and C diets were ground in a hammer mill with 1.0mm screen sieve size (mean particle size (MPS); resistant starch content = 328±1.6um; 0.11%; 392±1.5um; 0.92%, respectively). The RS- and WS-based diets were ground with 0.8, 1.0 and 1.6mm screen sieve sizes (MPS and resistant starch contents = 349±1.6um; 0.38%; 401±1.7um; 1.36%; 495±2.04 um; 2.4%, respectively), totaling eight extruded diets. The experiment followed a randomized complete block design with four blocks of 16 cats and two cats per diet in each block, totaling eight replicates per treatment. Food was provided to maintain constant body weight. After 30d of diet consumption, fresh feces were collected max 15min after voiding, snap-frozen and stored at -80^{o}C until analysis. DNA was extracted and the V4 region of the 16S rRNA gene was amplified and subjected to Illumina sequencing. Data analysis was performed using QIIME 2. Linear discriminant analysis effect size (LEfSe) was used to identify bacterial taxa different between treatments.

Results and discussion. Cats readily consumed all diets. Cats fed RS1.6 had decreased alpha diversity indices vs. C (OTU, Shannon index and Chao1: $p<0.05$), with similar results among the other diets. Principal coordinate analysis based on weighted Unifrac distance metric showed significant clustering between RS1.6 and WS1.6, compared to RS0.8 and WS0.8, as well as BR and C ($ANOSIM_{Weighted}$: $R=0.147$; $p<0.05$). LEfSe detected differences in abundance from bacterial taxa ($\alpha=0.01$, LDA score>2.0) on several phylogenetic levels. The proportion of Enterobacteriaceae increased in the feces of cats fed C ($P<0.05$). RS1.6 and WS1.6 were associated with Veillonellaceae and Bifidobacterium, respectively, groups known to produce short chain fatty acids [3,4]. RS0.8 increased abundance of Lachnospiraceae, a group associated with intestinal health [5], and the butyrate producers *Blautia* and *Faecalibacterium prausnitzii* [6,7].

Conclusion. Carbohydrate source and sorghum particle size had a significant effect on the fecal microbiota. Sorghum-based diets were associated with an increase in beneficial bacterial groups, showing a potential benefit for the intestinal health of cats. C-based diet led to increased fecal Enterobacteriaceae, an undesirable bacterial group.

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Influence of intact seaweeds on fecal microbiota and apparent nutrient digestibility in healthy adult dogs

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Introduction: Seaweeds represent a source of bioactive compounds having recently drawn attention for their possible application as health-promoting ingredients [1]. In particular, their indigestible polysaccharides have appeared promising prebiotic substrates in several animal species [2,3]. The present study investigated for the first time in the dog the effects of dietary supplementations with intact seaweeds (brown algae such as *Ascophyllum nodosum* (AN), *Undaria pinnatifida* (UP), *Saccharina japonica* (SJ) and the red alga *Palmaria palmate* (PP)) on some fecal microbial parameters and apparent total tract digestibility (ATTD).

Animals, materials and methods: Ten healthy adult client-owned dogs were used in a 5 × 5 replicated Latin Square design to determine the effects of five diets: control diet consisting of an extruded complete dog food (CD) not containing significant amounts of soluble fiber; CD + AN; CD + UP; CD + SJ; CD + PP. Seaweeds were added at the dose of 15 g/kg of the diet. The CD contained silica at 5 g/kg as a digestion marker. Each feeding period lasted 28 d, with a 7 d wash-out in between. Feces were collected at 21 and 28 d of each period for chemical (dry matter, NH_3, fatty volatile acids and biogenic amines) and microbiological (total bacteria, *Firmicutes,* enterococci, lactobacilli, bifidobacteria, *Clostridium* cluster I, *E. coli* and *Faecalibacterium prausnitzi*; by qPCR) analyses. Feces excreted during the last five days of each period were collected and pooled in order to evaluate ATTD. Data were analyzed by General Linear Model (with diet as fixed effect and animal and period as random effects), followed by Dunnet test as post hoc test. Statistical significance was set at $p \leq 0.05$.

Results and discussion: The chemical parameters were not influenced by seaweeds, except for *n*-butyrate that showed a tendency to be higher when dogs received SJ (+48% than CD; $p = 0.08$). The increased fatty volatile acids production by intestinal microbiota represents a well -known positive effect previously reported for brown seaweeds (typically containing 257-380 g of soluble fiber/kg) [3] or their polysaccharide extracts (alginate, laminarins and fucoidans) [4]. Microbiological analyses did not reveal any significant effect by algal supplementations. Nonetheless, dogs receiving UP displayed a tendency towards a decrease of the fecal abundance of *F. prausnitzii* (0.65 *vs.* 1.20 $\log_{10}$ copies/ng DNA; $p = 0.09$), a strain considered to be beneficial for gut environment and frequently depleted in canine gastrointestinal diseases [5]. Concerning ATTD coefficients, the algal supplementations did not influence neither the dry matter nor protein or ash digestibility. In literature, conflicting results obtained from studies in pigs have been published in this regard [6].

Conclusion: The algal supplementations at 15g/kg of diet (a dose commonly used for prebiotics in dogs) failed to exhibit noticeable effects on the fecal parameters. Further studies investigating higher dietary inclusions of intact seaweeds or their polysaccharides extracts are needed to better understand the potential of these interesting marine resources in companion animal nutrition.

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Impact of high-grain feeding on faecal microbiota and plasma metabolites in early lactation cows

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Introduction. Feeding high-grain diets often results in subacute ruminal acidosis (SARA) and dysbiosis increasing the risk of systemic metabolic disorders. The aim of this study was to determine effect of high-grain feeding on the faecal microbiota, hindgut fermentation, and the plasma metabolome of early lactating dairy cows.

Animals, materials and methods. Twenty-four lactating Simmental cows (BW = 737 [3]. 90 kg, lactation number = 4.1 ± 1.9; mean ± SD) were enrolled in this study. Cows were first fed a baseline diet with 60:40 forage:concentrate ratio (DM basis) for 14 days, then switched to a 40:60 forage:concentrate diet for four weeks to induce a SARA challenge. Blood and faecal samples were collected on day 14 (Baseline), day 21 (SARA1), and day 42 (SARA2). Blood metabolome profiling was carried out using the Biocrates MxP® Quant 500 kit, a targeted metabolomics approach based on electrospray ionization-liquid chromatography-tandem mass spectrometry. The output was analysed using MetaboAnalyst 4.0. Faecal samples- were used for bacterial 16S amplicon sequencing using Illumina MiSeq paired ends technology and analysed using the QIIME pipeline v2020.2. Statistical analysis was performed using the MIXED procedure of SAS taking into account repeated measures on the same animal.

Results and discussion. During SARA1 and SARA2 cows had a higher rumen acidosis index than during baseline. Multivariate analysis showed a different serum metabolite composition during baseline and SARA challenge. Cows had higher blood concentrations of bile acids and triglycerides and lower levels of carboxylic acids during SARA1 than in baseline. In SARA2, the main changes occurred at the level of lysophosphatidylcholines and phosphatidylcholines. Valine as well as the related amino acids cystine and taurine together with lysophosphatidylcholine C26:0 and several phosphatidylcholines, were classified as important features for cluster separation. Faecal bacterial taxonomic analysis revealed decreased alpha diversity (Shannon; $p = 0.01$) and species richness metrics (observed OTUs; $p = <0.01$) during SARA challenge. At the phyla level, *Actinobacteria*, *Firmicutes*, *Planctomycetes* and *Spirochaetes* were also affected by SARA ($p \leq 0.01$). There was an increase in the relative abundance of *Actinobacteria* and *Spirochaetes* in SARA1, while *Firmicutes* and *Planctomycetes* decreased. The faecal pH ($p = 0.04$) decreased throughout the SARA challenge, while the SCFA concentration ($p = 0.02$) peaked during SARA1 challenge.

Conclusion. High-grain feeding elicited major changes in the blood metabolome, which differed with the duration of SARA challenge. Changes in the profile of SCFAs in faeces during the SARA may reflect changes in the microbial community structure and function as a result of differences in substrate availability. Combining findings of the metabolome analysis with faecal microbiota might help in developing biomarkers for diagnosis and better characterization of SARA in cattle.

Effects of increasing rye levels in compound feeds on fermentative processes in the alimentary tract of young pigs

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Introduction: Especially the unique non-starch-polysaccharide fraction of rye (e.g. high levels of arabinoxylans and fructans) is one of the reasons for growing interest in this cereal. By microbial degradation of the above-mentioned carbohydrates higher levels of lactic acid in small intestine and butyrate in the hind gut with diverse beneficial effects might occur (gut health/animal welfare/prevalence of salmonella) [1]. Thus, this study was performed to determine potential effects of rye on concentrations of lactic and butyric acid in digesta of young pigs, according to increasing dietary rye levels. **Animals, materials and methods:** Forty pigs (age: 46.8 ± 5.28 days; bw: 16.1 ± 4.13 kg) were housed individually in four groups of 10 pigs each. Each group was fed a diet consisting of wheat and/or rye, barley, soy, potato protein and a mineral supplement. The sum of wheat and rye was 69 % in all diets, whereby the compound feed of each group was characterized by a different ratio (%) of wheat to rye (wheat/rye: 69/0; 46/23; 23/46; 0/69). After 4 weeks of dietary treatment, the pigs were dissected. In digesta samples, lactic (enzymatically) and butyric acid (gas chromatographically) concentrations as well as pH-values of small intestines digesta were determined. Statistics were done by SAS® (Anova).

Results and discussion: With higher dietary levels of rye there were increased concentrations of lactate and butyrate in the digesta. Moreover, pH tended to be lower in small intestines' digesta with higher dietary rye levels (group 1: 6.46 ± 0.739; group 2: 6.16 ± 0.602; group 3: 6.16 ± 0.481; group 4: 5.76 ± 0.679).

Table 1: Absolute lactic (g/kg) and butyric acid (mmol/kg) concentrations as well as relative (mmol/100 mol volatile fatty acids, VFA) butyric acid concentrations (original substance) in digesta of the young pigs (g = group; ± = SD)

g	lactic acid (g/kg)				butyric acid (caecum)	
	stomach	small intestine	caecum	colon	mmol/ kg	mmol/ 100 mol VFA
1	0.320 ± 0.298[a]	3.82 ± 3.17[a]	1.79 ± 2.19	0.436 ± 0.986	17.2 ± 0.80	11.1 ± 3.80
2	0.383 ± 0.757[a]	4.77 ± 3.02[ab]	1.91 ± 1.88	0.217 ± 0.602	20.1 ± 0.88	14.0 ± 3.60
3	0.931 ± 0.541[a]	4.68 ± 2.18[ab]	1.92 ± 2.22	0.532 ± 0.963	23.2 ± 1.03	13.6 ± 4.83
4	1.870 ± 1.460[b]	6.43 ± 2.68[b]	3.68 ± 3.48	1.590 ± 2.800	19.4 ± 1.08	13.5 ± 5.46

Conclusion: With higher dietary rye levels there was an intensified accumulation of lactic and butyric acid in the digesta. However, particularly high concentrations of lactate seem to affect the formation of butyrate in the content of caecum and colon (group 4), probably due to changes in the microbiota, as it was also shown in previous investigations [4]. On the one hand, antimicrobial effects of lactate and butyrate against gram-negative bacteria (e.g. *salmonella*) can be assumed [2/3], on the other hand with lower pH-values of small intestines' content due to particularly higher dietary rye levels in the diet, there could occur negative effects on the efficiency of prececal starch digestion in young pigs.

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Effect of type of cereals (wheat and wheat with rye) on fermentation processes determined in-vitro in canine model

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Introduction: Rye contains more biologically active compounds, such as dietary fiber, fructans, polyphenols and alkylresorcinols and anti-nutritive substances (protease inhibitors and α-amylases) and hybrid rye contains significantly lower mycotoxins compared to other cereals. Health-promoting effects of phytonutrients contained in rye grain have been proven in many human and animals (cattle, swine, poultry) studies. Therefore, the aim of this study was to determine the effect of replacing wheat grain with hybrid rye grain in dog food on palatability and feed intake, fecal quality and in-vitro fermentation parameters.

Animals, material and methods: The experiment lasted 20 days and was conducted on 12 dogs (Beagle at age of 2-7 y, kept individually) fed with a balanced dry food (composed of: extruded cereal grains, poultry meal, poultry fat, rapeseed oil, corn starch, dried eggs, tricalcium phosphate, calcium carbonate and premix for dogs). Dogs were divided into 3 groups based on diet: control (C) with wheat only, 80% wheat and 20% rye (E1) and 60% wheat and 40% rye (E2). Faeces quality and feed intake was measured from 14th day, 7 days after transition onto new food. In vitro gas production (GP) was estimated using gas test (glass syringes) and the fermentation parameters such as: pH, SCFA, BCFA, lactic acid and ammonia concentration, and degradability of organic matter dOM were determined [1]. Chemical composition of dog diets and faeces were evaluated by standard analytical methods [2]. Faeces of dogs in 14th day of experiment were mixed with phosphate buffer as inocula and incubated after adding dry food. Fermentation parameters were determined before and after fermentation (that lasted 24 h). Results were statistically analyzed using one-way analysis of variance and Pearson's correlation [3].

Results: Initially, the duration of the experiment has been planned for 45 days. However, after 20 days, we observed gradual deterioration of the quality of faeces in both experimental groups with rye, thus we decided to stop the experiment. In addition, from the 17th day, the consumption of feeds was decreasing. The faeces of dogs fed control diet had a higher pH (pH = 6.26) compared to the experimental groups 5.92 (E1) and 5.67 (E2) *(p=0.43)*. Maximum gas production and organic matter degradability were lowest in fermentation of control diet. Lactic acid production (35 mmol/L) during 24 hour fermentation was the highest for experimental diet with the highest proportion of rye (E2) (*p=0.30*). In addition, butyric acid production was twice as high in the experimental groups (about 7.4 mmol/L) compared to the control group (4.5 mmol/L) (*p=0.05*). The highest BCFA (*p=0.64*) and ammonia (*p<0.01*) production was observed in the control group.

Conclusion: Too high proportion of hybrid rye in dog food adversely affected food intake and faecal quality. However, a hybrid rye in dog food had a positive effect on the fermentation process according to in vitro studies. An accurate determination of the level of rye in pet food that could improve fermentation in the dog's digestive tract and lack negative effect on health of digestive tract and whole organism should be examined in a separate study.

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Session 3B Toxins, minerals II

Chair: Marta Hervera

Acrylamide analysis in dry pet food

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Introduction. Acrylamide is a potential carcinogenic and processing contaminant formed when determined feedstuffs, in particular those especially rich in starch and asparagine, are prepared at temperature above 120°C and low humidity.[1,2] Aim of this study was to assess if dry pet food can be a product at risk for this contaminant.

Material and methods. Eight complete pet food extruded dry diets (7 for dogs and 1 for cats) from the same manufacturer were tested for acrylamide and gelatinized starch levels using HPLC/MS method and via enzymatic titration, respectively. For acrylamide, limit of detection (LOD) was 30 µg/kg while limit of quantification (LOQ) was 100 µg/kg. As introduced in Table 1, the diets were divided into 4 categories depending on the protein content and grain inclusion. Main source of starch for grain free products was potatoes while rice was the starch source for grain based diets (except for the cat diet in which was wheat).

Results and discussion. Results are presented in Table 1. Acrylamide levels were below LOQ (i.e. < 100 µg/kg) for all the tested diets. Besides, the manufacturer confirmed that all the diets were extruded at temperature above 120 °C. These levels suggest a relatively low concentration of acrylamide in these dry pet diets. There is only another report on pet food levels of acrylamide in which researchers found the contaminant concentrations ranging from 106 to 358 µg/kg for dog food and from 66 to 269 µg/kg for cat food.[3] Interestingly, in our study traces of acrylamide were found in all the no grain products while only one of the grain-based diet had similar results. However, levels of gelatinized starch appeared to be independent from acrylamide levels.

Table 1. Levels of gelatinized starch and acrylamide in the chosen pet food products

	CP > 29%	CP ≤ 29%	CP > 29% No Grain	CP ≤ 29% No Grain
Fish-based	GS = 26,7±1,9 A = traces	GS = 27,0±1,9 A = nd	GS = 28,5±2,0 A = traces	GS = 23,0±1,6 A = traces
Poultry-based	GS = 24,9±1,8 A = nd	GS = 32,3±2,2 A = nd	GS = 23,7±1,7 A = traces	GS = 25,9±1,8 A = traces

CP = Crude Protein; GS= Gelatinized Starch (g/100g); A = Acrylamide; nd = not detectable; traces = LOD<x<LOQ

Conclusion. The acrylamide content in these samples was below the risk levels of exposure determined in humans[1] even in high temperature extruded diets. However, we should also consider that pets, unlike humans, are usually fed only one kind of commercial diet, with little variation in the daily intake. Therefore, even traces of this contaminant may carry a risk and further studies should be developed.

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Effect of fatty acid composition, time and storage conditions on the lipid oxidation of fish-based and meat-based dry dog foods

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Introduction. Lipid oxidation (LO) constitutes a major risk for pet food preservation [1,2], as it causes substantial sensory alterations [3,4] and the consumption its by-products can negatively affect animal health [5,6]. Aim of this study was to investigate the effects of storage time, container type and product type (fish-based, FB, *vs* meat-based, MB) on fatty acid (FA) composition and LO of dry dog food.

Materials and methods. Seven complete dry dog foods, of which four FB and three MB, were supplied by four manufacturers and produced in the four weeks prior to analysis. The packages were opened for the first sampling after the delivery (T0); for each of them, half of the content was kept in the original package whereas the other half was transferred in a plastic bin. Both containers were stored in the same unlit room at 20-25°C, and further samples were collected after two (T1), three (T2) and six months (T3). Proximate analysis, FAs profile, conjugated dienes detection (K232, a measure of primary products of LO) and thiobarbituric acid reactive substances assays (TBARs, a measure of secondary products of LO) were performed for each sample. Data were submitted to descriptive analysis, ANOVA and Spearman correlation (r). Statistical significance was set at $p < 0.05$ and $r = \pm 0.60$.

Results and discussion. As expected, FB products were characterized by higher levels of omega-3 FAs (p=0.0001) and lower levels of saturated FAs (p=0.016) than MB ones. At T1, kibbles stored in plastic bins showed higher levels of TBARs than those in the original packages (p=0.0048); no differences were detected afterwards. At each time, TBARs of FB pet foods were significantly higher than those of MB ones (p=0.0005). TBARs were not affected by time in MB pet foods but increased significantly in the FB ones at T3 (p=0.0003). Therefore, FA composition seemed to have a stronger influence on LO than storage time. Product type did not affect K232 values, but a significant effect of time (p<0.0001) was observed as they increased up to T2 and then dropped at T3, probably due to the transformation of primary products of LO into secondary ones. Significant positive correlations emerged between TBARs and total polyunsaturated FAs content (r=+0.62) and omega-3 FAs content (r=+0.72); TBARs and saturated FAs content (r=-0.74) were inversely correlated.

Table 1. LSMEANS ± SE of TBARs and median and interquartile range of K232 of FB and MB pet food (data of bins and original packages samples combined).

Time	TBARs (mg/kg), FB	TBARs (mg/kg), MB	K232, FB	K232, MB
T0	1.9 ± 0.2^{a}	0.8 ± 0.2	6.5 (5.8 – 7.3)^{a}	5.9 (5.7 – 6.1)^{a}
T1	2.0 ± 0.1^{a}	1.0 ± 0.2	16.6 (11.8 – 18.9)^{b}	14.3 (10.8 – 18.8)^{b}
T2	2.1 ± 0.1^{a}	1.4 ± 0.2	23.0 (20.8 – 29.0)^{c}	23.3 (22.2 – 26.2)^{c}
T3	2.7 ± 0.1^{b}	1.1 ± 0.2	6.2 (5.9 – 6.3)^{a}	5.9 (5.6 – 6.4)^{a}

Conclusion. In this study, pet food FA composition had the highest impact on LO. Particular care should be taken when preserving products rich in omega-3 FAs, which LO rate was also affected by storage time. It seems therefore preferable to consume FB kibbles quicker and to store them in their original package rather than plastic bins, since this accounted for a better preservation during the first two months.

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Effects of deoxynivalenol and lipopolysaccharide on kidney health parameters in broiler chickens

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Introduction. The *Fusarium*-mycotoxin deoxynivalenol (DON) potentially interferes with performance and the immune system. Earlier studies showed that DON increases water intake (WI) in chickens and rabbits, potentially indicating effects on kidney health [1;2]. Lipopolysaccharides (LPS) are part of the outer membrane layer of Gram-negative bacteria and stimulate the innate immune system [3]. The aim of this study was to investigate the impact of DON and LPS on kidney weight and kidney health parameters in serum of broiler chickens.

Animals, material and methods. A total of 160 broilers were either fed a basal diet (CON), or a basal diet artificially contaminated with 10 mg DON/kg feed (DON) from day 1 of life. One day before sample collection, half of the birds received an LPS challenge (1 mg LPS from *E. coli* O55:B5/kg body weight) via crop gavage, while the remaining birds received distilled water (placebo; 2 × 2 factorial arrangement). Birds were sampled in week 3 (d19-23, n=72) and 5 (d33-37, n=80) of the experiment, respectively. Kidney weight was recorded and serum samples were analysed for uric acid, albumin, total protein, calcium (Ca) and phosphorous (P). Statistical analysis was performed with PROC MIXED of SAS (SAS Inst. Inc., Cary, NC). All animal procedures were approved by the institutional ethics committee of the University of Veterinary Medicine Vienna and the Austrian national authority (GZ 68.205/0062—WF/V/3b/2015); [4].

Results and discussion. In 3-week-old chickens fed the DON-diet with oral LPS-challenge, relative kidney weight increased by 14% compared to DON-fed chickens without LPS-challenge ($p < 0.05$). This potentially indicates an acute swelling and inflammatory response in DON-fed chickens in the presence of an additional inflammatory stimulus. However, this effect was only present in 3-week-old birds but not in 5-week-old chickens [4]. Therefore, DON and LPS may affect earlier stages of immune and kidney development. In 3-week-old chickens, serum Ca decreased by 4.5 and 3.5% in the CON-group with LPS challenge and DON-group with placebo-challenge, respectively, compared to birds receiving the CON-diet with placebo challenge ($p < 0.05$). Serum albumin and total protein were unaffected by the treatments ($p > 0.10$) in week 3 and 5. Serum uric acid increased by 21% in LPS-challenged 3-week-old chickens compared to their placebo-challenged counterparts (p
[4]. 0.05). Serum P decreased by 5% in LPS-challenged 5-week-old chickens compared to the placebo-challenged chickens ($p < 0.05$).

Conclusion. This study shows that both DON and LPS interfere with kidney health parameters in growing broiler chickens. Further research unravelling the underlying mechanisms of DON and LPS on kidney health is required.

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Phytogenic feed additive reduced ammonia and odor emissions in weaned piglets

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Introduction. Fecal gas emission became one of the major concerns arising with the intensification of swine production within the past few years. Ongoing research is focused on developing feed additives able to improve the performance and overall health of animals and at the same time decreasing a negative impact on the environment. Supplementation with phytogenic feed additives (PFA) has the potential to reduce unpleasant odor and toxic gases in livestock production [1]. Phytogenic feed additive containing a special blend of essential oils, active compounds hereof and other extracts was tested to reduce odor and ammonia emissions in weaned piglets.

Animals, material and methods. A total of 321 weaned piglets (York x Tempo) were divided into 2 groups, each with 6 replicates and 26 animals per replicate. The pigs in the control group (CON) were fed a basal diet, while pigs in the PFA group were supplemented with a phytogenic feed additive (Digestarom® DC Xcel, BIOMIN, Austria) at 150 g/t feed. Diet composition of the starter and grower phase met the requirements of national nutritional standards (Netherlands). Odor emissions were determined on days 7, 21 and 42 according to the European/Dutch standard NEN-EN 13725. The levels of ammonia were monitored every 6 hours from day 9 to day 42 using sensors (Dol-sensor a/s) and calculated as an average value for each day. The growth performance of the pigs was evaluated for the total period of 5 weeks.

Results and discussion. Supplementation with PFA resulted in a 39 % and 48 % reduction of odor on days 21 and 42 respectively in comparison to the CON group. The average ammonia emissions within the last 17 days of the trial were reduced by 23.1 1) in PFA group when comparing to CON group. Taking into consideration ammonia emission data, ventilation records and the number of animals, annual ammonia emission was calculated in the current trial. This calculation revealed that annual ammonia emission per animal place was decreased in the PFA group about 26.2 % compared to the CON group in the period of the last 17 days of the trial. In general, diet supplementation with phytogenics can increase feed digestibility and enhance immune response of the gut [1, 2]. Better utilization of the nutrients together with improved intestinal health can contribute to the lower fecal gas emissions observed in the current study. Average daily weight gain was about 5.7 % higher in the PFA group with 4.5 % increase of the final body weight on day 42 in comparison to the CON group. Phytogenic feed additive tested in the current study was designed to support the digestion and improve the performance of piglets that is in line with the observed results.

Conclusion. The supplementation with phytogenic feed additive in weaned piglets extensively reduced ammonia and odor emissions and improved growth performance.

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Apparent digestion of total and highly soluble phosphate in adult dogs fed different phosphate sources

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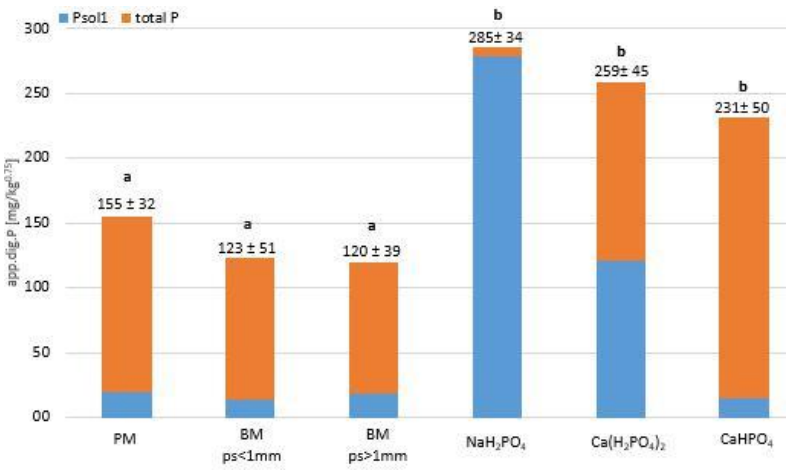
Objectives: Numerous studies demonstrated adverse health effects of oral phosphorus (P) excess in humans[1-3] and animals[4-7], especially when highly soluble inorganic P sources (Pi) were used. Because solubility is a precondition for absorption, the solubility and therefore source of P is thought to affect the apparent digestibility of the element. The aim of this study was to determine the amount of apparently digested total and highly soluble P (after 1 minute in water (P_{sol1})) and its effect on serum P concentrations in dogs fed excess P from different sources.

Animals, materials & methods: Eight healthy Beagles (3±1y; 14±1kg BW) were consecutively fed 6 high P (HP) diets aiming at the fivefold recommended daily allowance (RDA[8], P ~2.1% DM). The HP diets contained either solely organic phosphates (poultry meal (PM), bone meal (BM) of different particle sizes (ps;<1mm; 1-3mm, Ca/P=1.9)) or had Pi added (NaH_2PO_4, $Ca(H_2PO_4)_2$, $CaHPO_2$; Ca/P=1.4). To obtain the targeted Ca/P of 1.4/1 and 1.9/1, respectively, $CaCO_3$ was added when needed. Each diet was fed in a single meal for 18 d (13d adaptation, 5d balance trial), followed by a wash-out period of ≥14d. During the balance trial, faeces was collected quantitatively. Blood was sampled on day 18. Feed, faeces and serum were analysed for P (photometric, modified vanadate molybdate method[9]). P_{sol1} was determined in feed and faeces applying the method of Lineva et al. (2018)[10]. The apparently digested amount of total P and P_{sol1} as well as the AUC over 7 hours of serum P (sP)[5] were calculated. The proper authorities for animal welfare approved the study. Statistical methods: Descriptive statistics, One Way RM ANOVA.

Fig. 1: Apparently digested P [mg P/kg $BW^{0.75}$] from 6 HP diets (mean±SD)



Results & discussion: Diets with supplemented Pi contained higher amounts of P_{sol1} than diets with organic P ($p<0.001$). The solubility of the tested P sources P_{sol1} was different (PM, BM < $CaHPO_4$ < $Ca(H_2PO_4)_2$ < NaH_2PO_4) while concentrations of Pi and P_{sol1} in a diet were correlated (R^2= 0.43). Adding Pi salts to a diet increased the amount of apparently digested total P significantly (Fig.1; $p<0.05$) even in the less soluble Pi source $CaHPO_4$. The percentage of apparently digested P_{sol1} was highest in NaH_2PO_4 (53±9%; PM: 22±5%; BM <1mm: 21±8%; BM >1mm: 21±4%; $Ca(H_2PO_4)_2$: 42±4%; $CaHPO_4$: 41±6%). The sP concentrations, determined as AUC sP[5], were increased in all HP diets containing Pi salts ($p<0.001$). Highly soluble Pi sources (NaH_2PO_4, $Ca(H_2PO_4)_2$) caused an earlier and steeper increase of sP[5] than the less soluble Pi source $CaHPO_4$. In order to predict the impact of P intake on a dog's P homeostasis and health, the source and solubility of P has to be considered.

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A 30 week feeding trial to aid in establishing no observed adverse effect levels (NOAELs) for different sources of phosphorus in feline diets

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Introduction. High dietary phosphorus (P), particularly added as soluble salts, may contribute to the development of chronic kidney disease (CKD) in cats [1; 2; 3]. There is currently no guidance for safe maximum limits of P in cat foods. Consequently it is important to establish safety for different dietary P sources. The aim of this study was therefore to establish the safety of P supplied at 1g/1000kcal from a highly soluble P salt, at two different total P levels, in feline diets.

Animals, material and methods. Seventy-five healthy adult cats (n=25/group) were fed one of three dry, extruded experimental diets – a low P control (1.4g/1000 kcal; Ca:P 0.97) or one of two test diets with moderate (Test 1 - 4g/1000 kcal; Ca:P 1.04) or high (Test 2 - 5g/1000 kcal; Ca:P 1.27) total P – for a period of 30 weeks in a randomised parallel design. Due to the basal Ca and P content of chicken and pork meals in the diets, Test 2's Ca:P could not be adjusted to <1.27. Both test diets contained sodium tripolyphosphate (STPP) providing 1g/1000 kcal of total P. At baseline and at regular time points, blood, urine and faeces samples were collected, glomerular filtration rate (GFR) determined, and renal ultrasound and bone density (DXA) scans performed to assess health. Following transition to a dry commercial diet (total P – 2.34g/1000 kcal, Ca:P 1.3), further blood and urine samples were collected after 4 weeks. Twenty one cats had been on this diet during screening, while the rest (n=54) had received a commercial wet diet. Responses were analysed via linear mixed effects models and fixed effects of diet group, time point (weeks) and the interaction between diet group and time point.

Results and discussion. There was no significant effect of diet on GFR, DXA or ultrasound findings and all blood and urine parameters remained within physiological reference ranges. However, urea, creatinine and fibroblast growth factor-23 (FGF-23) significantly increased in cats fed test diets from week 2 onwards ($p<0.001$, $p\leq0.041$ and $p\leq0.028$, respectively). All cats showed a positive P and Ca balance, however this was significantly increased by ≥3 fold in the test diet groups compared to the control group at week 27 ($p<0.001$). On transition to the commercial diet for 4 weeks, serum creatinine and urea values for the two test diet groups decreased to levels below or equal to those observed during screening, indicating these were transiently influenced by diet differences during the test period.

Conclusion. The present data suggest NOAELs for the dietary P levels in the tested feline diets, specifically 1gP/1000 kcal from STPP and total phosphorus level of 4 or 5g/1000 kcal (Ca:P 1 and 1.3, respectively) after 30 weeks of feeding. Differences in physiological parameters observed during the trial were considered regulatory responses needed to maintain mineral homeostasis and adaptations to dietary factors other than P levels. These data will assist industry, regulators and professional bodies in developing guidance on safe maximum dietary levels of P for healthy adult cats.

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A case of suspected vetch poisoning and trifoliosis in dairy cattle

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Case history: In a dairy farm with ~100 German Black Pied cattle, 4 cows had to be euthanized due to severe signs of alopecia, salivation and reduced general condition. The pathological examination led to the suspicion of vetch poisoning. Vetch poisoning of cattle is a generalised disease characterised pathologically by infiltration of skin and many internal organs by monocytes, lymphocytes, plasma cells, often eosinophils, and multinucleated giant cells and clinically by dermatitis, pruritus, often diarrhoea, wasting and high mortalitiy[1]. The animals were housed in tie-stalls and fed a total mixed ration (TMR) produced at the farm. The TMR contained a basis of 30% corn silage, 10% hay, 30% grass and 30% of the so-called 'Landsberger Gemenge', a mixture of ryegrass *(Lolium perenne*), crimson clover (*Trifolium incarnatum)* and hairy vetch (*Vicia villosa*). Each cow received barley, rapeseed meal extract and a mineral supplement in different amounts in addition to the TMR, to accomplish individual requirements. At least temporarily, the farmer increased the amount of 'Landsberger Gemenge' in the TMR to 50-60%.

Materials & Methods: Firstly, all components of the TMR were analysed for feed value and feed hygiene by sensorial inspection. Because of suspected poisoning by plant material, a macroscopic and microscopic botanical examination was carried out. Because of the detected abnormalities of the clover, a microbiological examination (microscopy and morphological analysis of mycological culturing) was initiated.

Results & Discussion: The sensorial inspection showed an average feed value and hygiene quality of all TMR components. The 'Landsberger Gemenge' contained an above average amount of vetch. This plant contains potentially toxic proteins (lectines). From literature is known, that the above-mentioned signs can be experimentally induced in cattle by feeding 9 kg of vetches per day for less than 2 weeks[1]. In the actual case, large quantities of vetches have been fed (calculated intake of >9kg vetches from 15-30 kg 'Landsberger Gemenge' per day). The microbiological examination performed because the bottom sides of the cloverleaves were covered in black spots (Fig. 1), revealed the fungal *Cymadothea trifolii* that causes reproductive dysfunctions due to coumestrol, a substance with oestrogen-like effects, as well as skin lesions and ulcera (trifoliosis).

Fig. 1: Clover (bottom side)

Conclusion:

Both vetch poisoning and infestation with *Cymadothea trifolii* might have been responsible for the reported problems in the herd. We recommended to take measures to reduce the percentage of vetch in the 'Landsberger Gemenge' and to ensure that the clover is not infested with *Cymadothea trifolii* in future cultivation.

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Calcium, phosphorus, zinc and copper content in commercial supplements for horses

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Introduction. To ensure adequate diets that meet the nutrient requirements of horses, owners rely on correct declarations on feed and supplements. Severe over or under supply of macro- and micronutrients may result in health issues. The aim of this study was to determine possible discrepancies in the manufacturers' declaration regarding calcium, phosphorus, copper and zinc concentrations in commercial supplements for horses.

Materials and methods. Samples of 19 randomly selected commercial mineral feed products for horses were analysed in duplicates for their crude ash, calcium, phosphorus, copper and zinc content at the Institute of Animal Nutrition, Freie Universität Berlin, according to the the VDLUFA methodologies. The results were compared to the declared values. The complementary feed products where manufactured by different producers and comprised products easily accessible for German horse owners on the internet. Copper, zinc, and calcium were analysed with atomic absorption spectroscopy (AAS, contrAA 700, Analytic Jena AG, Germany). Vanadate molybdate method was used to determine the concentration of phosphorus at 436 nm (Ultrospec™ 2100 pro Amersham Biosciences, Amersham Biosciences Europe GmbH, Germany, modified after VDLUFA 10.6.1). Results were compared to the declared values considering the tolerance range and crude ash content [1,2].

Results and discussion. Declaration of trace elements differed between total and added content depending on the manufacturer. Considering the analysed mineral concentrations, 18 out of 19 samples showed deviations from the declared values above or below the tolerance range [1] in at least one analysed parameter. There was only one product without discrepancies. Of the 18 complementary feed products which differed more or less from the declaration, nine of calcium, nine of phosphorus, seven of copper and seven of zinc concentrations were within the tolerance range. For calcium, two samples presented lower (22-63 %) and eight samples presented higher (11-45 %) concentrations, as declared by the manufacturer. Phosphorus was lower (10-66 %) as declared by the manufacturer in eight samples, while an excess (10-101 %) was observed in two samples. The calcium/phosphorus ratio based on the declared concentrations ranged from 1.4:1 to 12:1 and from 1.5:1 to 6:1 when analysed values were considered. Nine analysed copper concentrations were below (15-71 %) and three were above (41-342 %) the declared values. The same picture arose for zinc with nine lower (20-51 %) and three higher (17-804 %) analysed concentrations, as compared to the declaration. Despite the described discrepancies, recommended feeding rates assured adequate supply of calcium, phosphorus, and cupper for a 500 kg horse fed hay from Berlin Brandenburg. However, 16/19 complementary feeds did not supply sufficient zinc. Additionally, 14 samples contained vitamins.

Conclusion. This study has shown deviations between the declared and analysed mineral concentrations in the analysed feed samples. Thus, regular and independent quality controls of supplements for horses are advised.

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The effects of feeding varying concentrations of phosphorus and zinc to laying hens on the apparent ileal and total tract digestibility of phosphorus and zinc

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Introduction. Earlier published data showed efficient hydrolyzation of dietary phytate in chickens fed low phosphorus (P) [1]. This may affect utilization of dietary zinc (Zn) [2]. The present study investigated interactions between high and low concentrations of dietary P and Zn on apparent digestion of P and Zn in layers.

Animals, Materials and Methods. 48 adult layers (0.93 eggs/hen*day^{-1}, 65 g/egg) were kept on sawdust in 24 boxes housing each one Lohmann Brown and one Lohmann White hen (body weight 2134 ± 222 g and 1809 ± 82.1 g). The study comprised 14d of acclimatization and 8d of experiment. During acclimatization, animals were fed according to recommendations (AMEn 11.4 MJ/kg, XP 20.8%, Lys 1.23%, Met 0.37%, total Ca 38g/kg, total Zn: 130mg/kg) but P supply was varied (P(-): 3.67 g/kg; P(+): 8.42 g/kg; CaH4P2O8). For experimental feeding, P levels were kept and further combined with varying Zn (Zn(-): 27 mg/kg, Zn(+): 130 mg/kg; ZnSO4*7H2O). 0.1% TiO2 was used to estimate apparent ileal (Pi, Zni) and total tract (Pf, Znf) digestion. Feces was collected animal-wise on d8 and animals were slaughtered to collect ileal chyme (last 20 cm). Data analysis comprised 3-way ANOVA (P, Zn, breed) and interactions (P≤0.05).

Results and Discussion. Performance and body condition were not affected. P(-) feeding decreased Pi and Pf significantly (PP < 0.0001 for both). The latter was further negatively affected by Zn-feeding in combination with P(-) (PZn=0.05, PZn*PP=0.08). P(-) and Zn(-) negatively affected Zni and Znf independently of each other (PP=0.07 and 0.05, PZn=0.02 and 0.04).

Table 1. Digestibility of P and Zn in layers fed varying levels of P and Zn.

	P(+)		P(-)		SEM	P-value		
	Zn(+)	Zn(-)	Zn(+)	Zn(-)		P	Zn	Breed
Pi, g/d	0.19	0.23	-0.20	-0.09	0.17	<0.0001	0.19	0.34
Pf, g/d	0.15	0.16	-0.23	-0.06	0.15	<0.0001	0.05	0.85
Zni, mg/d	-4.00	-2.88	-9.07	-3.22	4.90	0.07	0.02	0.34
Znf, mg/d	-6.52	-10.0	-9.90	-11.6	4.30	0.05	0.04	<0.0001

P(+), 8.42 g/kg dietary P; P(-), 3.67 g/kg dietary P; Pi and Zni, ileal P and Zn digestion; Pf and Znf, fecal P and Zn digestion; SEM, standard error of means; Zn(+), 130 mg/kg dietary Zn; Zn(-), 27 mg/kg dietary Zn.

Conclusion. Very low numerical values were presumably due to sawdust uptake, allowing only relative comparisons. Previous data from this project [3] showed bone mobilization due to P(-) feeding and associated bone Zn losses and subsequent increase in circulatory Zn. This may have initiated downregulation of active Zn absorption in P(-) groups to counter regulate increased circulatory Zn, which has yet to be proven by further measurements.

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Selenium retention in dog food during extrusion processing

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Introduction: Selenium (Se) is added to pet food formulations through its regular sources as sodium selenite (Na_2SeO_3) or selenium-yeast (Se-Y). Non-published studies from our laboratory found that there is a difference among Se sources in selenium retention in diets after extrusion, which is not described for pet food. The objective of the present study was to evaluate Se retention along the extrusion process of a dog food formulation supplemented with Na_2SeO_3 or Se-Y.

Materials and methods: A control diet based on corn, poultry by-product meal, soybean meal, and poultry fat was formulated to dog maintenance, and a mineral-vitamin supplement without Se was added (CON). This diet was supplemented with 400 or 800 µg of Se/kg using Se-Y (Selplex; Alltech Agroindustrial, Brazil), purified anhydrous Na_2SeO_3 (P_{NaSe}; Sigma-Aldrich Products, Brazil), or Na_2SeO for animal feed (C_{NaSe}; Incasa, Santa Catarina, Brazil), totaling 7 experimental diets. After mixing, the ingredients were ground and extruded in a single-screw extruder (MEX-250, Manzoni, Campinas, Brazil). The processing conditions were not changed among diets: preconditioner mass temperature 89.8 ± 4.2ºC; mass temperature (124.6±1.7ºC), and pressure (28.8±17.4 Mpa) before extruder die; in-barrel moisture 22.7±0.7%; kibble apparent density 369±24g/L; specific mechanical energy application 11.0±2.5kWh/ton. Kibbles were then dried in a forced air dryer at 125^{o}C for 25 min. After system stabilization, samples were collected at 15-min intervals from extruder and dryer, with a minimum of 4 replicates per treatment (experimental unit). Se was analyzed with the graphite furnace atomic absorption spectrometry technique in an atomic absorption spectrometer (model AA-6800, Shimadzu, Tokyo, Japan). All analyses were conducted in triplicate and repeated when the coefficient of variation was greater than 5%. The results were subjected to analysis of variance of repeated measures (raw materials x after extrusion and dryer) and means compared by the Tukey test (P<0.05).

Results and discussion: Se in CON was not affected by processing (P<0.05). A minimal decrease on Se-Y was observed only at 800 µg/kg inclusion (7% decrease; P<0.05), but not at 400 µg/kg, with recovery rates ranging from 93% to 104% (DM basis, comparing final values with raw mixture). For both Na_2SeO_3 sources, lower concentration was observed after extrusion and drying, with recovery rates ranging from 62% to 86% and a mean loss of 23.4% (P<0.01). It is suggested that the temperature, shear, pressure, and moisture during extrusion might convert part of sodium selenite into hydrogen selenide, lost as a gas.

Analyzed selenium concentration (DM-basis)

Item	Inclusion µg/kg	Raw Mixture	Extruder	Dryer	P-value
CON	0	38±0.73	39±0.73	40±0.73	0.206
Se-Y	400	387 ^{b} ±7.0	414 ^{a} ±6.1	416^{a}±6.1	0.028
	800	829^{a}±5.3	795^{b}±5.2	804^{b}±6.0	0.005
C_{NaSe}	400	574^{a}±11.4	358^{c}±11.4	404^{b}±11.4	<.0001
	800	879^{a} ±11.7	754^{b} ±11.7	755^{b} ±10.1	<.0001
P_{NaSe}	400	559^{a}±7.6	382^{b}±7.6	386^{b}±7.6	<.0001
	800	898^{a}±4.6	760^{b}±4.6	775^{b}±4.6	<.0001

a, b – mean in a row without a common letter differ (P<0.05).

Conclusion: Se from selenium yeast was stable, whereas sodium selenite presented losses induced by the extrusion process.

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Environmental impact of commercial diets for dogs in Brazil

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Introduction. Food production is responsible for 26.0% of greenhouse gas emissions (GGE) and land use [1] and, therefore, its environmental impact should be considered for a sustainable system [2]. The hypothesis is that canine feed represents a considerable share of total gas emission and land use of Brazil. The aim of this study was to evaluate the environmental impact of dry and wet commercial diets for adult dogs in Brazil by estimating the land use and the emission of carbon dioxide equivalents (CO_2eq).

Animals, material and methods. A total of 372 diets, of which 294 were dry and 78 were wet, were selected. According to label information regarding guaranteed analysis and ingredients, the inclusion percentages of each ingredient of each diet was estimated using a formulation software (Optimal 2000, Campinas, Brazil). Based on the inclusion percentages, the land use and CO_2eq emission were calculated and annual estimates considering the feed intake of a single dog and consequently the entire Brazilian canine population (52.2 million) [3] were performed.

Results and discussion. The mean land use was 0.78±1.81 hectares and the mean CO_2eq emission was 2.75±5.44 tonnes per dog per year. As for the environmental impact of the entire dog population in Brazil, a total of $40.49x10^6$±$94.80x10^6$ hectares/year and 0.14±0.28 gigatonnes CO_2eq/year were estimated, which is equivalent to 10.0% of the total emission of Brazil in 2016 or the emission of 20.9 million Brazilian citizens [4]. Wet diets were significantly more responsible for land use and emission ($p<0.0001$) (Figure 1). The results are similar to previous studies in Japan, China, and the Netherlands [5] and lower than estimates for the U.S. [6].

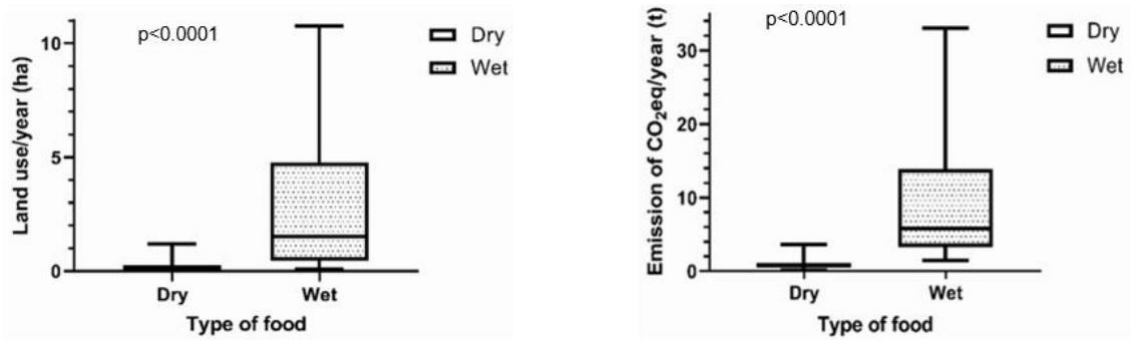

Figure 1. Use of land and emission of carbon dioxide equivalents (CO_2eq) of one dog, considering the food intake of one year for either dry or wet commercial diets.

Conclusion. A great part of environmental impact generated by food production, human or animal, is caused by the use of ingredients of animal origin. Results of this study suggest that a substantial part of the environmental impact is generated by canine feeds and should not be discarded in the discussion of sustainability. Solutions include higher inclusions of plant-based ingredients or the search for alternative ingredients, such as insects.

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Owner perceptions of health for dogs fed plant-based or meat-based diets

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Introduction. A previous investigation reported that 2% of dogs are fed plant-based diets[1], though there is little research regarding their suitability. Previous studies have revealed nutrient deficiencies in a number of plant-based (PB; devoid of all animal products) pet foods[2], though dogs eating plant-based diets have not been reported to have demonstrable nutrient deficiencies or adverse health outcomes attributable to diet[3,4]. It was hypothesized that dogs fed PB diets would have higher prevalence of disorders potentially associated with nutrition, such as ocular, urinary and cardiac health conditions.

Animals, material and methods. An e-survey designed using Qualtrics software was distributed to customers of pet food retailers and promoted through social media platforms. Multiple choice and short answer text entry questions included pet owner demographics, general dog management, health, and nutrition. The study was approved by the institutional research ethics board (REB # 18-07-039). Descriptive data were reported as frequencies and percentages, chi square analyses were used to compare differences between diet groups.

Results and discussion. Of a total of 1,413 respondents, 916 had dogs only, 497 had dogs and cats. Dog sex was evenly distributed, with 50% female and 50% male. Most (89%) were desexed. Mean dog ownership was 1.7 dogs per respondent, ranging from 1 to 13. Of the 1,189 respondents who reported dog diet, 665 (56%) were meat-based [MB], 357 (30%) were PB, 63 (5%) were a combination of the two [combo], and 104 (9%) were indeterminable. Median dog age was 6 years (r. 4 months to 18 years). No difference in age was found between diet groups. Dogs were owned for 5.0 years (r. 0-18). Overall, dog owners considered their dogs to be in very good (941, 71%) to good (320, 24%) health. More dogs fed PB (283/356, 79%), than MB (459/665. 69%) were considered to be in very good health ($P = 0.001$). Owner-reported body condition score (BCS) ranged from 1 (15, 1.5%) to 9 (30, 2.2%) on a 9-point scale, with 4-5 being ideal. Most respondents (776/1,343, 58%) indicated their dog to be in ideal body condition, and mean BCS was 5.2. Obesity was reported for 3.7% (48/1,313) of dogs, correlating with the number of dogs having a body condition score > 7 (2.2%). There was no association between diet and BCS. Over half of dogs had at least one health disorder (789/1,413, 56%), with a mean of 1.0 disorders per dog (r. 0 to 7). Diet was not associated with number of reported health disorders. Most frequently reported health conditions were dermatological (269/1318, 20%), dental (254, 19%), otic – including dermatological (175, 13%), gastrointestinal (144, 11%), musculoskeletal (113, 8.6%) and ocular (112, 8.5%). There was no association between diet and prevalence of any health disorder.

Conclusion. Owners of dogs fed plant-based diets do not report more health disorders than owners of dogs fed meat-based, and perceive their health to be better than do owners of dogs fed meat-based diets.

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Session 3C Obesity

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Dietary choline supplementation mobilizes hepatic lipids and increases lipoprotein transport in obese cats

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Introduction. Obesity is a major health concern for the feline population. Weight loss is recommended in overweight cats, yet current weight-loss programs tend to have disappointing outcomes and are not without risk. Severe energy restriction predisposes obese cats to primary hepatic lipidosis [1]. As choline is linked to lipid metabolism [2], it was hypothesized that dietary choline supplementation would assist in the elimination of fat from the liver through increased lipoprotein transport.
Animals, Material and Methods. This study was approved by the University of Guelph Animal Care Committee (AUP#2494). Twelve colony cats with naturally occurring obesity (body condition score [BCS]:≥8/9 [3]) were split into two groups. Following a four-week adaption period, cats were fed a control diet (n=6; 4,587mg choline/kg DM) or a high choline diet (n=6; 18,957mg choline/kg DM) for five weeks at maintenance energy requirements (NRC:130 kcal/kg $BW^{0.4}$ [4]). Both diets had the same ingredient and nutrient profile, apart from the additional choline chloride. Body weight and BCS were assessed weekly. On day 0, and at the end of the five-week testing period (day 35), fasted blood samples were taken, and body composition was assessed by dual energy X-ray absorptiometry (DEXA). Serum was analyzed for cholesterol (CHOL), high-density lipoprotein CHOL (HDL), triglycerides (TG), non-esterified fatty acids (NEFA), glucose (GLUC), and insulin. Very low-density lipoprotein CHOL (VLDL) and low-density lipoprotein CHOL (LDL) were calculated [VLDL=TG/2.2; LD=CHOL–HDL-C–VLDL] [5]. Plasma amino acids (glycine, alanine, valine, leucine, ornithine, methionine, phenylalanine, citrulline, tyrosine) were also analyzed. A repeated measures ANOVA, with time as the within subject factor and diet as the between subject factor, was used. A Bonferroni post-hoc test was performed to assess multiple comparisons. Statistical significance was set at $p<0.05$.
Results and discussion. Body weight, BCS and body composition remained unaltered throughout the study ($p>0.05$). Supplemental dietary choline resulted in increased serum CHOL, TG, HDL, LDL, VLDL and plasma methionine ($p<0.05$). Serum GLUC tended to decrease with choline supplementation ($p=0.051$). There were no changes in serum insulin or the other amino acids analyzed ($p>0.05$).
Table 1. Serum lipid profile (mmol/L) of obese cats fed control diet (control) (n=6) compared to high choline diet (choline) (n=6) from day 0 to day 35

	Control–d0	Control-d35	Choline-d0	Choline-d35	p-value
CHOL	5.64 ± 0.38	5.61 ± 0.30	4.92 ± 0.21[a]	5.98 ± 0.20[b]	0.013*
TG	0.53 ± 0.033	0.50 ± 0.026	0.48 ± 0.070 [a]	0.58 ± 0.054 [b]	0.038*
HDL	5.02 ± 0.30	4.93 ± 0.25	4.51 ± 0.13[a]	5.21 ± 0.16[b]	0.029*
NEFA	0.42 ± 0.034	0.52 ± 0.027	0.42 ± 0.046	0.41 ± 0.038	0.090
LDL	0.38 ± 0.11	0.45 ± 0.10	0.19 ± 0.11[a]	0.50 ± 0.14[b]	0.014*
VLDL	0.11 ± 0.0067	0.10 ± 0.0052	0.097 ± 0.014[a]	0.12 ± 0.011 [b]	0.038*

Values expressed as mean ± SEM; [ab]Values with superscripts without a common letter differ; *p< 0.05; d0,day 0; d35,day 35

Conclusion. These findings suggest an effect of choline assisting in eliminating hepatic fat through increased fat mobilization and enhanced methionine recycling.
Acknowledgments. Funding from NSERC, in partnership with Elmira Pet Products.
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"What? A weight loss program for obese cats?" - A survey with the owners' perspective

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Introduction. Owners frequently underestimate the body condition score (BCS) of their obese cats[1]. Achieving the cat's ideal body weight (BW) is a way to increase its lifespan[2] and avoid comorbidities[3]. To increase the owner's compliance for a successful weight loss program (WLP) it is important that they understand its benefits and identify changes in their cat's quality of life (QoL).

Animals, materials and methods. After a WLP (3-6 months) in 25 privately-owned obese cats (BCS≥7/9), a survey assessing the owners' perception of the cat's BCS, QoL, activity level, behavior and main difficulties was undertaken. Prior to the WLP a complete anamneses was done, investigating the cat's current diet and owners were asked to identify their cat's BCS (1-9/9). During the WLP all cats were consistently fed a dry weight loss diet at 293kJ/kg ideal BW and kept indoors. Also, owners were advised to increase the activity of their cats. Weight and food intake were recorded weekly and daily, respectively. The food was readjusted by ±10% if the weight loss (WL) was not between 0.5-2%/week. Owners that didn't have the same answer in a duplicated question were considered unreliable and were excluded. 19 questionnaires were then evaluated. Spearman correlation coefficients (r) were analysed using SPSS v25.0 software to explore the relationship between the WL and the data from the questionnaire. Assessment of the BCS by veterinarians and owners was compared.

Results and discussion. Before starting the WLP, the BCS (mean 8.3/9) was under and overestimated by 37% (n=7) and 11% (n=2) of the owners, respectively. 94% (n=18) of the cats lost weight and the average WL was 14.2% (-0.2 to 28.6%) at a mean energy of 0.84 x RER (0.64 to 1.13) and a mean BCS of 7.5/9 by the end to the WLP. Owners reported that the overall QoL of their cats was unchanged (26%; n=5) or increased (74%; n=14) after the study. The perceived QoL improved by 20% after the WLP. Interestingly, the perception of the QoL was correlated to the WL ($r=0.46$, $p<0.05$), and a real decrease in the cats' BCS was correlated to the owners' perception of this change ($r=0.71$; $p<0.01$). Increased activity levels were reported by 73% (n=14), while the average of activity level for those cats that had a WL>15% (52%; n=10) was 48% higher than the ones that lost less than 15% (48%; n=9). The main difficulties during the WLP were "not feeding extra food" (32%; n=6) and "limiting the cat's access to other types of food" (26%; n=5). 53% (n=10) believed that the main difficulty for the cat was "to have its food reduced over the time". Although 68% (n=13) of the owners reported not giving treats prior to the study, all of them described some difficulties in not giving extra food. This reinforces the role of optimal communication since the owner might not identify table scraps as treats. Despite several difficulties, the vast majority of owners (95%; n=18) believed the cat was "happy" during the WLP.

Conclusion. Owners could visually identify changes in the cat's BCS. The reduction in BW was related to the owners' perception of QoL.

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Impact of dietary fibre properties on satiety in cats

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Introduction. Overweight in Western pet cats causes unnecessary health risks and is unfortunately common. Solution strategies could involve diets that limit overeating, and this makes it important to understand how dietary factors impact on food consumption. Fibres are known to stimulate satiety in humans according to physicochemical properties [1-3] such as gastric gel formation (e.g. alginate; ALG), intestinal viscous gel formation (e.g. psyllium; PSY) or large intestinal fermentability (fructooligosaccharides; FOS). How these fibre properties relate to satiety in cats and affect post-meal inhibition or food motivation is presently unknown. We addressed this by testing the effects of fibre properties in cats' daily meals on food motivation using a model of operant conditioning.

Material and methods. Food motivation was measured repeatedly in 8 healthy adult cats using operant conditioning. Cats were trained to press a lever for a standard dry extruded food reward in a progressive ratio schedule that determined the maximum effort they were willing to make. Depending on fibre type, motivation for food was tested exactly at 3, 5 or 8 h after the start of the morning meal. For the morning meal, a commercial low-fibre extruded food (crude fibre 0.6 g/MJ ME) was combined with a wet cat food (0.3 g/MJ ME) without fibre (control; CON) or mixed with ALG, PSY or FOS. Daily dosage of each fibre was based on effective dosages for appetite suppression reported in humans [1-3], extrapolated to cats on a metabolic body weight (BW) basis (75 to 4 kg). Dietary ALG, PSY and FOS contents were 0.18, 0.91 and 0.71 g/MJ ME contributing 41, 79 and 74% of total fibre. Foods with fibre were fed in the morning (7:45-8:45, 40% of ME requirements for maintenance) and the dry food without additions in the afternoon (16:45-17:45, 60%). Fibre effects were tested with 8 healthy adult cats. Fibres potentially suppress appetite at different moment after the meal, which were assumed to be around 3 h for ALG, 5 h for PSY and 8 h for FOS. Each fibre was tested against CON in a cross-over design with two periods each with 2 d of adaptation and testing on day 3 after the morning meal. Food intake was recorded throughout the study and the cats' BW before and after the study. Data were log-transformed. Per fibre type, data were analysed by a mixed model in SAS with Diet (CON, fibre), Period (1 or 2) and Sequence (1 or 2) as fixed effects in the model and cat as the random effect.

Results and discussion. The daily food intake throughout the study was almost 100% and all cats remained healthy. Six cats lost some BW (<5%) and two gained BW (<2%). Feeding cats ALG or PSY did not affect food motivation (P>0.10; Figure 1). Cats fed FOS obtained on average 11.8 rewards, which was less than 14.3 rewards for CON (P=0.004). The appetite suppressing potential of FOS raises questions about its effects under conditions of ad libitum food intake. For ALG and PSY, dosages may need to be increased to prolong satiety.

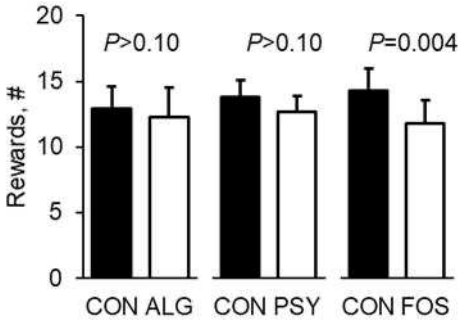

Figure 1. Food rewards obtained by lever pressing of cats (n=8; mean+SE) after a morning meal without (CON) or with alginate (ALG; test at 3 h post meal), psyllium (PSY; 5 h) or fructooligosaccharides (FOS; 8 h).

Conclusion. FOS supplementation was found to suppress appetite in adult cats whereas ALG and PSY did not.

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GO TO ABSTRACT LIST

Quantification of fecal short chain fatty acids in lean and obese client-owned dogs: preliminary results

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Introduction. Obesity in pets is a growing nutritional disease [1]. It has been suggested that enhanced capacity to harvest energy from food, especially via carbohydrate fermentation and thus, short chain fatty acids (SCFA) production, could be related to obesity [2]. Most of the available studies evaluate the impact of dietary interventions on SCFA's fecal concentrations in research dogs [3,4]. The aim of this study was to characterize fecal concentrations of SCFAs in lean and obese client-owned dogs.

Animals, material and methods. Fifteen lean (LD) (Body Condition Score (BCS) = 5 on a 9-point scale [5]) and 28 obese (OD) (BCS ≥ 7/9) privately-owned adult Labradors and Golden Retrievers were recruited for this study and declared healthy based on clinical examination, blood biochemistry and complete blood count. Fecal samples were obtained from overnight fasting dogs fed their usual diets (that were documented in their nutritional history). Each sample was homogenized and, within 15 minutes, frozen at -80°C until analysis. SCFA concentrations were determined by gas chromatography coupled to mass spectrometry. Fecal pH was measured with a portable pH meter and stool samples were lyophilized to determine dry matter (DM). Statistical analyses were performed with an independent sample *t* test for normally distributed variables and a Mann–Whitney U test for non-normally distributed variables; results are expressed as mean (±SD) or median (first quartile – third quartile).

Results and discussion. Dogs were: 23 females (17 neutered) and 20 males (14 neutered). The mean body weight of LD and OD were respectively 30.2±4.5kg (BCS of 5), and 40.3±7.0kg (mean BCS of 7.8±0.6). The percentage of DM was respectively in LD and OD's feces 71±5%, and 68±4% (p=0,017); and the pH 6.5±0.5 and 6.6±0.4 (p=0,4) . OD, compared to LD, presented higher fecal concentrations of SCFA. Differences, partially presented in table 1, were significant for butyrate (C4), isobutyrate (iC4), and isovalerate (iC5), and not significant for acetate (C2), propionate (C3), valerate, hexanoate, heptanoate and octanoate.

Table 1. Concentrations of fecal SCFA in OD & LD (expressed in µmoles/g DM)

	C2	C3	iC4	C4	iC5
OD	93.6 (±30.6)	78.2 (±25.1)	2.9 (2.3-4.3)	22.8 (±8.5)	4.0 (2.9-5.7)
LD	86.4 (±35.8)	70.7 (±26.8)	1.8 (1.0-3.0)	14.3 (±6.1)	2.5 (1.2-4.2)
p-value	0.52	0.40	0.032	0.003	0.027

The accumulation of C4 is often positively associated with the intake of fiber [6]. A third of the OD's usual diets were "light" pet foods, which generally contain a slightly higher concentration of fiber than "standard" ones. This could partly explain this difference. Also, abundance of major butyrate-producing bacterial groups has already been reported to increase in obese dogs [7]. In order to clarify the role of obesity per se, a future trial will look at differences between LD and OD fed with the same diet.

Conclusion. This study suggests that fecal concentrations of butyrate, isobutyrate and isovalerate can be higher in obese dogs, maybe due to the diet they receive.

Reference: [1] German (2006), *J. Nutr.*, 136 (7), 1940S-1946S; [2] Turnbaugh *et al.* (2006), *Nature*, 444 (7122), 1027–1031; [3] Alexander *et al.* (2018), *Br. J. Nutr.*, 120 (6), 711–720; [4] Bosch *et al.* (2009), *Br. J. Nutr.*, 102 (2), 318–25; [5] Laflamme (1997), *Canine Pract.*, 22 (4), 10–15, [6] Duncan *et al.* (2007), *Appl. Environ. Microbiol.*, 73 (4), 1073–8; [7] J. Xu *et al.* (2017), *BMC Vet. Res.*, 13(1), 374.

Female and mixed breed dogs have greater difficulty for weight loss

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Introduction. Obesity is considered the most common nutritional disorder in dogs and responsible for the occurrence of significant comorbidities and it is a risk factor for the development of many chronic diseases [1]. However, the evaluation of the effect of different variables (gender, sex status, age, body size, and breed) for its treatment, although very important, is still little explored. The objective of this study was to analyze the weight loss program, assessing the dynamics of weight loss of different groups.

Animals, material and methods. From a database of 1,053 cases, assessed between 2012 and 2019 at the routine of the Veterinary Nutrology Service - Veterinary Hospital of the FMVZ/USP, São Paulo, Brazil, 77 cases of obese dogs (body condition score of 8/9 or 9/9 [2]) of different ages, breeds, body size, gender, and sex status were selected. These dogs didn't have any concomitant illnesses and successfully completed the weight loss program, followed according to NRC guidelines (2006) [3]. Statistical analysis was performed with the aid of PROC MIXED from the SAS program, version 9.3. Values of $p \leq 0.05$ were considered significant.

Results and discussion. Regarding reproductive status (p=0.797), age (p=0.473), and body size (p=0.150) there were no differences between groups, allowing the same calorie intake per kg of body weight $(BW)^{0.75}$ to be used in intact and neutered dogs; dogs with 1 to 8 years old and older than 8 years old; and for dogs of different body sizes. As for the gender variable, some studies have evaluated the effect on weight gain [4, 5] but not on weight loss. In our study, the average calorie intake for weight loss was is 64.07 and 66.60 x $BW^{0.75}$ for females and males, respectively (Figure 1), significantly different (p=0.005). And finally, regarding the breed, the calorie intake required for weight loss was lower in mixed breed dogs (63.13 x $BW^{0.75}$) when compared to pure breed dogs predisposed to obesity (65.05 x $BW^{0.75}$) and other pure breeds (66.41 x $BW^{0.75}$) (Figure 2) (p=0.001).

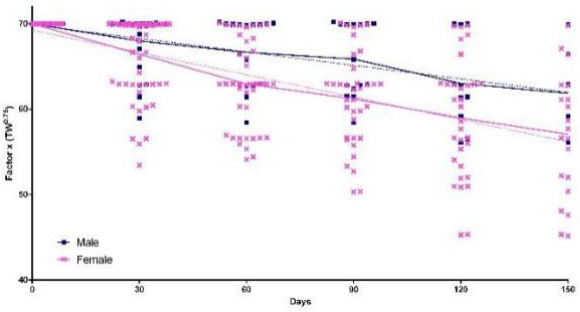

Figure 1. Complete distribution of the factor applied to the weight loss equation [factor x $(TW^{0.75})$] as a function of the animal's sex.

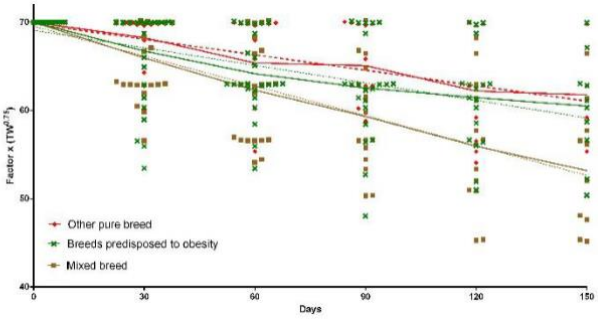

Figure 2. Complete distribution of the factor applied to the weight loss equation [factor x $(TW^{0.75})$] according to the breed of animals.

Conclusion. It was concluded that females and mixed breed dogs have greater difficulty in losing weight and requires greater negative energy balance during the weight loss program.

References: [1] German et al. 2006 J. Nutr.136:1940-1946. [2] Laflamme, D.P. 1997 Canine Pract. 22: 10-15, 1997. [3] NRC. 2006. Nutrient requirements of dogs. [4] Mao et al., 2013 Prev. Vet. Med. 112:438-442. [5] Usui et al., 2016 Asian Pac. J. Trop. Biomed. 6:338–343.

A96

Evaluation of leptin and antioxidant contents in canine serum and milk samples: preliminary data

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Introduction. Leptin is one of the most important hormones produced and secreted by adipocytes, which is involved in food intake and energy homeostasis [1]. Leptin is also synthesized by mammary gland and detectable in milk [2]. The aim of this study was to evaluate and compare the content of leptin and Total Antioxidant Capacity (TAOC) in repeated serum and milk samples collected at different times during lactation in dogs. **Animals, material and methods.** Ten healthy bitches of different breeds were included in the study. Serum and milk samples were collected at delivery (T0), 7 days (T1) and 21 days (T2) *post-partum*. Furthermore, analysis of milk macronutrients composition was performed. The obtained data were analysed using IBM SPSS Statistics V. 25 software [3]. **Results and discussion.** During lactation leptin content both in serum and milk samples showed a progressive slightly reduction. In particular, at T0 serum leptin was 3.98 ± 0.78 ng/ml, at T1 was 2.75 ± 0.8 ng/ml and at T2 was 2.54 ± 0.38 ng/ml. Leptin concentration in colostrum (T0) was 2.07 ± 0.71 ng/ml, at T1 decreased to 1.74 ± 0.14 ng/ml, and at T2 was 1.65 ± 0.2 ng/ml. Serum leptin concentration was significantly higher than milk at T2 ($P<0.05$). Similar results have been described in pregnant women [4]. In serum TAOC concentration was quite constant during lactation, ranging from 6.20 ± 0.11 to 6.28 ± 0.08 µmol Trolox equivalent/L. In milk TAOC content at delivery was 3.65 ± 0.43 µmol Trolox equivalent/L, then slightly increased to 4.20 ± 0.29 µmol Trolox equivalent/L at T1, and re-decreased to 3.36 ± 0.45 µmol Trolox equivalent/L at T2. In human literature TAOC values of mature milk were reported slightly lower than those of colostrum and transitional milk [5]. Serum TAOC concentrations were higher than milk at T2 ($P<0.05$). Milk macronutrients composition in colostrum showed lipid (10.05 ± 4.34 g/100g) and protein (6.32 ± 0.41 g/100g) high concentrations which decreased at T1 (8.63 ± 3.34 g/100g, 5.78 ± 0.45 mg/ml, respectively) and slightly increased again at T2 (10.28 ± 2.51 g/100g, 6.07 ± 0.19 mg/ml, respectively), even though without a significant difference. Carbohydrate concentration was lower (6.45 ± 1.88 g/100g) in colostrum than in milk at T1 and T2 (7.04 ± 2.93 g/100g and 7.35 ± 2.29 g/100g, respectively). In agreement with others studies [4], leptin and TAOC concentrations both in serum and milk samples did not show any correlations with the type of *partum* (natural delivery or caesarean section), and the BCS of the mothers. **Conclusion.** These data represent a preliminary report on leptin and TAOC concentration in serum and milk of canine species, which confirms the potential usefulness of these two matrices for diagnostic purposes. Further studies are needed to prove their role on clinical aspects involved in bitch fertility and pup health, mainly in weight management to prevent obesity.

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Preliminary study: voluntary activity of overweight and non-overweight cats evaluated by accelerometer 3-axyal

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Introduction: Voluntary activity in cats is little studied[1] with few information regarding the implications of body composition, reproductive status or diet[2]. It is possible that overweight cats may be less active, with lower energy expenditure. This preliminary study compared the voluntary activity of overweight and non-overweight lab cats.

Animals, materials and methods: Eight neutered laboratory cats were divided (approval protocol 9536/18) into two groups: Overweight (O; n=4; 5.5±0.8 years; 5.1±0.3 kg; body condition score [BCS] 7.7±0.9 of 9); Non-overweight (NO; n=4; 2.7±2.2 years; 3.2±0.5 kg; BCS 5.0±0.0). Both cat groups were housed in a collective cattery of 46.6 m^2, equipped with suspended shelves, scratching posts, and a solarium. They were fed *ad libtum* a dry commercial food (maintenance diet of the cattery) replaced at 11:00 and 16:00 h, and were daily stimulated to play by the keepers from 09:00 to 10:00 h. Cats were conditioned to chest collars for 7 days, and then a 3-axial accelerometer (AC; Axy-4, TechnoSmart, Italy) attached for 3 consecutive days. The AC was configured at a frequency of 10 Hz, 16 G gravitational scale and 10bit resolution. Data of the x, y, and z axes were smoothened for each second and then subtracted from the corresponding unsmoothed data to produce a value for G, resulting from the dynamic acceleration. Derived values were then converted into absolute positive units and the resultant values from all three channels were summed to give an overall dynamic body acceleration (ODBA)[4]. Force[5] was calculated [F (N) = acceleration (m/s^2) x body mass (kg)]. The mean values of the 3 days of each cat were used to calculate: maximum ODBA (ODBAmax), ODBA summation (ΣODBA), area under the curve (AUC) of ODBA, number of peaks (NP) above 1 G, total area of the peaks (TAP). The preliminary results were compared by Student's T test ($P<0.05$).

Results and Discussion: Overweight cats showed lower ODBAmax (4.5±0.6G) value than NO animals (5.9±0.5 G; $P<0.01$; Fig 1). The ΣODBA value was similar between the two groups (O: 4419±216 G; NO: 4525±447 G; $P=0.4$). The mean (O: 2.5±0.3N versus NO: 1.7±0.2 N) and maximum F value (O: 227.5±31.9N versus NO:186.2±32.4 N) were higher for overweight cats ($P<0.01$), explained by their higher body mass that require more F and energy to move at a given distance[5]. The ODBA AUC (O:4644±481G/s; NO:4590±442G/s), NP (O:37±6; NO:38±13), and TAP (O:1778±478 G/s; NO:1529±672 G/s) did not differ between groups ($P>0.05$).

Conclusion: Overweight cats showed similar activity levels than NO cats, but lowest maximum overall dynamic acceleration of movements. The implication of this differences on energy expenditure need to consider the higher force they need to move their mass.

Acknowledgements: Affinity Pet Care to support the study.

References: [1]Nutrient Requirement Council of Cats and Dogs (2006); [2]Backus R & Wara A (2016) doi.org/10.1016/j.cvsm.2016.04.002; [3]Laflamme D. (1997) Canine Pract, 22, 10–15; [4]Wilson, RP. et al. (2006) doi:10.1111/j.1365-2656.2006.01127.x; [5]Moore WJ. Físico-química, v. 1. Editora Blucher, 1976.

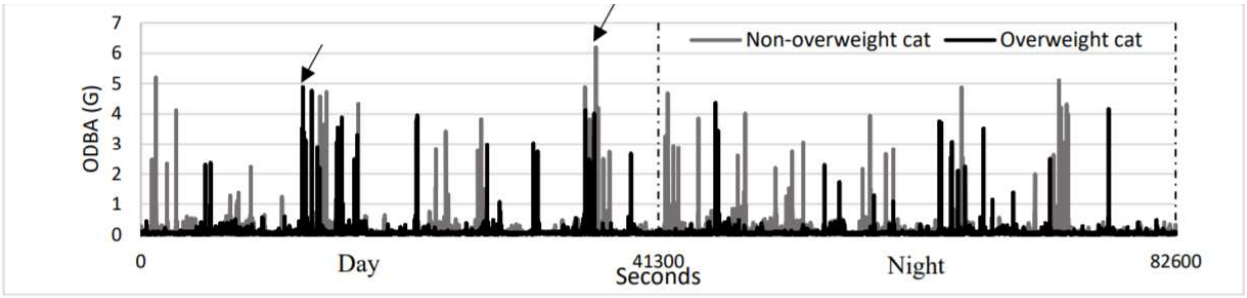

Fig 1: ODBA results of one overweight cat and one non-overweight cat. Arrows show the highest peak of each animal.

GO TO ABSTRACT LIST

Interactions between microbiota, diet and obesity in client-owned dogs: preliminary results

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Introduction. Obesity in pets is a growing nutritional disease [1]. In human, gut microbiota composition plays a role on the development of obesity and its comorbidities [2,3]. The aim of this study was to investigate the impact of obesity and a specific diet on dogs' fecal microbiota composition.

Animals, material and methods. Fifteen lean (LD) (Body Condition Score (BCS) = 5 on a 9-point scale [4]) and 28 obese (OD) (BCS ≥ 7/9) privately-owned adult Labrador and Golden Retrievers were recruited for this study and declared healthy based on clinical examination, blood biochemistry and complete blood count. In order to limit the influence of diet on the results, all the dogs received for one month the same diet whose composition was (% Dry Mater): proteins 35, nitrogen-free extract 33, lipids 11, metabolizable energy 300kcal/100g (NRC06). Daily metabolizable energy requirement (MER) was estimated according to dog diet history when known, or using the NRC 1974 formula MER = 132 x initial $BW^{0,75}$ with a 0.7 correction factor when neutered. Fresh fecal samples were collected at enrollment (V1) and 1 month later (V2), homogenized, stored in a DNA stabilizer, and frozen at -80°C until extraction and sequencing of microbial DNA, and amplicon profiling analysis. A Mann-Whitney U test and a Wilcoxon rank sum test with a false discovery rate were performed to examine significant differences respectively, between groups and between visits regarding alpha and beta diversity, and population abundance. Results are expressed as mean (±SD) or median (first quartile – third quartile).

Results and discussion. Two dogs (1LD and 1OD) lost weight, probably due to underestimation of their previous diet, and were excluded. Dogs were: 22 females (16 neutered) and 19 males (13 neutered). The mean BW of LD and OD were respectively 30.6±4.5kg (BCS 5) and 40.6±7.0kg (mean BCS 7.8±0.6). Microbiota composition in OD and LD, at V1 and V2, are partially presented in table 1.

Table 1. Evolution of the core microbiota at phylum level between V1 and V2 in OD and LD (expressed in relative percentage – no statistical significance).

	OD at V1	Delta V2-V1 OD	LD at V1	Delta V2-V1 LD
Bacteroidetes	34.1 (25 - 59)	13.5 (-12.6 - 23.1)	67.7 (45 - 75)	5.3 (-11.0 - 26.8)
Fusobacteria	29.5 (7 - 48)	-2.8 (-22.0 - 6.5)	14.3 (7 - 22)	-0.7 (-9.2 - 4.0)
Firmicutes	20.2 (5 - 30)	1.1 (-8.4 - 6.2)	10.8 (6 - 24)	3.3 (-6.3 - 10.4)

Giving the same diet to all the dogs appeared to result in less variation in microbiota populations, without having a drastic loss of richness or diversity. The high individual and interindividual diversity and the evolution in the core microbiota were in accordance with previous studies [5]. Moreover, the impact of diet seemed, albeit not significant, to be stronger in OD, whose microbiota has been previously suggested to be less stable [6]. This study is still in progress and tends to clarify the role of diet, obesity, and energy restriction on the fecal microbiota structure.

Conclusion. This study failed to show a statistically significant influence of obesity or diet on dog's microbiota.

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Age dependency of free activity in dogs

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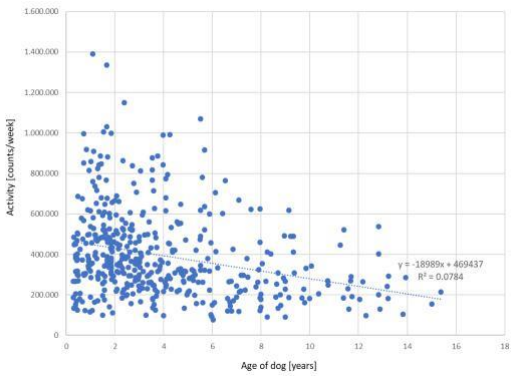
Introduction: Energy requirements of dogs are age dependent and decline when the animal grows older[1]. It is commonly accepted, that this is at least partly due to a decline of spontaneous activity with age, independent of the health status. However, evidence-based information about this assumption is sparse. The aim of this study was to gather data about free activity in healthy dogs of different age and to evaluate the results regarding a possible age effect.

Animals, material and methods: Up to now, 454 dogs between the age of 4 months and 15.4 years and a body weight ranging between 6 and 45 kg (BCS present only in a subset of dogs) were available for the study. Of these dogs, 142 were privately owned, the rest kennelled. A precondition for the participation in the study was a good general health status or rather the absence of acute disease with possible effects on the activity level. For ~3 weeks the dogs were carrying omnidirectional accelerometers (Actical, Philips Healthcare; 32counts/sec, 1min epoch length) attached to their collars. Dog owners were asked not to alter their daily routine with the dog. The correlation between mean counts per week and age of the dogs was tested. Activity in different age groups was tested using the Kruskal-Wallice One Way ANOVA on Ranks. Ethical approval number 95-13-09-2017).

Results and discussion: The number of old dogs suitable for this survey was limited because diseases become more frequent and prevented the admission to the study. As expected, the overall weekly activity level showed a huge variation (77500 to 1.4 Mio counts; Figure 1).



The correlation between activity and age was slightly more pronounced in privately kept dogs but overall it was very weak (R^2=0.0784). Therefore, the activity level alone does not allow to draw any conclusions regarding age. However, the range of activity is smaller in dogs ³8 years when compared to younger animals, the activity significantly lower ($p<0.001$). In this survey, the median activity in the group of dogs of 8 years and above was 65% of the median activity level measured in the group of younger dogs (<8 years).

Fig. 1: Activity counts/week in 454 dogs aged 0.3 to 15.4 years

Conclusion: Healthy dogs of older age show an amount of free activity also detected in younger dogs. However, activity above ~400k counts per week occurred more seldom in this age group (10 vs. 42% of observations). We hypothesise, that in dogs a decline in activity plays a role in the reduction of energy requirements with age. To avoid obesity in older dogs, the energy supply should be adjusted to match varying individual needs. To ensure a sufficient nutrient intake, the nutrient density in a food or ration might have to be increased. More research is warranted to define the age effect more precisely.

Reference:

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Modified gene expression in visceral adipose tissue of young Beagle dogs is associated with adult obesity

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Introduction. Altered gene expression of adipose tissue has been proposed as one possible mechanism for excess fat mass deposits in the development of obesity. An early altered transcription of proteins involved in energy metabolism, insulin sensitivity, lipolysis, lipogenesis, adipogenesis and thermogenesis could be predictive factors for development of obesity in later life. The study aimed to identify differences in gene expression in adipose tissue in a population of young dogs, some of whom spontaneously developed obesity in adulthood.

Animals, material and methods. Twenty-four female Beagle dogs (birth date ± 4 weeks) were raised in the same kennel environment and were presented with the same diet for 3.5 h per day in unlimited quantities, in a protocol approved by the Royal Canin Committee for Animal Ethics and Welfare and the Animal Use and Care Advisory Committee of Pays-de-la-Loire. During the sterilization procedure at 8 months of age, subcutaneous and visceral adipose tissue samples were collected. The mRNA levels of genes involved in lipolysis (perilipin, HSL, LPL), lipogenesis (ACC, ADRP, CPT1, FAS, FABP), adipogenesis (PPARγ, SREBP-1c), thermogenesis (UCP1, UCP2), glucose uptake (GLUT4) and insulin sensitivity (adiponectin, IRS2) were estimated by real time RT-PCR. When the dogs reached 24 months of age, they were allotted *a posteriori* to 2 groups according to their body condition score (BCS): ideal weight (IW, BCS=5 and 6, n = 13) and overweight (OW, BCS=7 and 8, n = 9). Gene expression levels in the 2 groups were calculated by the 2^{-DDCt} method and compared using the Mann Whitney test. If residuals were not normally distributed, variables were transformed as appropriate.

Results and discussion. No significant difference in BCS at 8 months of age was detected. Thirteen of the 14 genes examined were expressed at lower levels in the visceral adipose tissue of dogs in the OW compared to the IW group. Of these, 4 were significantly lower: LPL (median [range] 0.07 [0.00-0.92] vs 2.74 [0.02-9.39]), FABP (0.64 [0.18-1.25] vs 1.04 [0.37-1.71]), PPARγ (0.63 [0.14-1.36] vs 1.05 [0.43-2.47]) and GLUT4 (0.63 [0.21-1.36] vs 1.31 [0.31-1.60]) (all $p<0.05$; $q=0.14$). Modification of gene expression was not observed in subcutaneous adipose tissue. A lower expression of PPARg, and its downstream genes (Glut4 and LPL) in visceral adipose tissue in obese and insulin-resistant adult dogs has been previously reported [1].

Conclusion. For the first time, this study shows a modification in gene expression in visceral adipose tissue of puppies that could potentially be linked to their disposition to develop obesity in adulthood when fed *ad libitum*. The causal link between altered gene expression and obesity remains to be investigated.

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Evaluation of the nutrient supply of prescription diets for obese dogs available in the Brazilian market

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Introduction. In a weight loss program, there is a risk of nutritional deficiency due to the energy restriction [1].Therefore, the aim of this study was nutrient analysis in prescription diets for obese dogs, and analyze whether under energy restriction for weight loss, these foods meet the minimum recommendations of these nutrients per kg of metabolic weight.

Animals, material and methods. Samples of eight prescription diets for obese dogs sold in the Brazilian pet food market were analyzed, corresponding all this sector of the Brazilian market. Nutrient analysis were performed according to AOAC [2]. The essential minerals determination was performed by inductively coupled plasma optical emission spectrometry (ICP-OES). The amount that an animal consumes of nutrients per kg metabolic weight through the analyzed foods was simulated, using the formula for weight loss: 70 kcal/target weight$^{0.75}$ (target weight= initial body weight minus 20%). The results were descriptively compared with NRC (2006) [3] and FEDIAF (2019) [4] recommendations.

Results and discussion. According to comparisons with NRC [3], all analyzed foods provided at least one nutrient below the minimum recommendation, 50.00% of them had three nutritional deficiencies, and 25.00% of foods had four nutritional deficiencies. The main deficient nutrients were: selenium (Se), copper (Cu), potassium (K), magnesium (Mg), and fat. According to comparisons with FEDIAF [4], 62.50% of the foods resulted in at least two nutritional deficiencies, and 25.00% in at least three nutritional deficiencies. The following nutrients were deficient: Se, K, Mg, and calcium (Ca). Decreased intake of these nutrients may cause organic changes. Se deficiency can compromise the antioxidant and immune systems, and Ca deficiency may result in secondary nutritional hyperparathyroidism [3]. Hypomagnesaemia is associated with increased vascular reactivity and increased vascular tone [5]. As for K deficiency, it has been observed that it can influence blood pressure and renal perfusion in adult dogs in the short term [6].

Conclusion. The commercial prescription diets for obese dogs available in the Brazilian market may result in deficiency of some nutrients for animals submitted to weight loss program. It is emphasized that if adjustments are made during the weight loss program, decreasing the amount of food if the animal does not lose the minimum weight stipulated, nutritional deficiencies can be even more alarming.

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Metabolomic fingerprinting of healthy dogs to reveal pathways in relation to high fat and high starch intake

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Introduction. High dietary fat intake has consistently been linked to increased rates of obesity in humans and animals alike [1,2]. However, to date, no study has investigated the effect of high fat diets on gastrointestinal metabolism and possible adverse health effects in dogs. The aim of this work was to map the gastrointestinal metabolome and altered metabolic pathways in dogs fed a high-fat vs. high-starch diet.

Animals, materials and methods. Ten healthy adult beagles of ideal body weight and condition (4.2±2.6 Y/O.; 10.5±1.2 kg) were equally allocated into two groups in a cross-over design. Each group received two diets, high-fat (HF; horse fat) and high-starch (HS; pregelatinized corn starch), for six consecutive weeks each, with a one-week adaptation in between periods. Diets were formulated to be isonitrogenous and isoenergetic, the HF and HS diet contained 18.2 g crude fat, 13.9 g crude protein, 0.2 g crude fibre and 5.0 g nitrogen-free extract per MJ and 5.2 g crude fat, 13.1 g crude protein, 0.3 g crude fibre and 35.0 g nitrogen-free extract per MJ, respectively. At the end of each period, faecal samples were collected and stored for UHPLC-HRMS based metabolomic analysis as described by De Paepe et al. (2018) [3]. Data processing was performed according to Rombouts et al. (2017) [4]. Two-way ANOVA, OPLS-DA modelling, and pathway analysis were processed for statistical analysis. A p value <0.05 was considered significant, while a p <0.10 was considered a significant trend. The study was approved by the local ethical committee (EC2012/112).

Results and discussion. A total of 16 metabolites could be retained, with 6 unidentified and 10 putatively annotated metabolites. Dogs fed a high fat diet displayed a higher abundance of leucyl-valine, karalicin and 5 non-annotated marker molecules, whereas dogs fed a high-starch diet displayed a higher abundance of L-methionine, leucyl-leucine, isoleucyl-threonyl-valine, valyl-leucine, isoleucyl-leucine, glycyl-valine, leucyl-serine, seryl-isoleucine, and 1 non-annotated marker molecule. Pathway analysis demonstrated a significant impact of high-starch diet on cysteine and methionine metabolism ($p = 0.042$), and a trend of impact regarding aminoacyl-tRNA biosynthesis ($p = 0.061$). Recent research has demonstrated that methionine intervenes in lipid metabolism and anti-oxidation [5,6], cysteine and aminoacyl-tRNAs play an important role in protein biosynthesis [7]. The result suggests a close association of these functions in relation to high starch diet.

Conclusion. The present study uncovered distinct differences in the faecal metabolome of dogs fed a high-starch vs. high-fat diet. The high-starch diet may promote lipid metabolism, anti-oxidation and protein biosynthesis compared to the high fat diet.

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Serum metabolomic profile in dogs with chronic kidney disease fed a renal diet

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Introduction. Chronic kidney disease (CKD) is highly prevalent in dogs [1]. Therapeutic diets are recommended to control and retard the progression of disease [1,2]. The aim of this study was to compare the serum metabolomic profile between healthy dogs and dogs with CKD in order to evaluate whether the diet intervention would have an effect on serum metabolites. **Animals, material and methods.** All procedures were approved by the Ethics Committee of the University of São Paulo (protocol # 3138/2013). Ten neutered dogs (5 females; 5 males, 8.9±4.5 y-old) with CKD stages 3 or 4 (IRIS classification) [4] were included in the CKD group. Therapeutic diet met maintenance requirements for adult dogs; however, the concentration of protein and phosphorus was reduced while ω-3 PUFAs and vitamin E were added. All dogs were in a normal diet and had a 4-day adaptation period to the new diet. The control group was composed of ten healthy neutered dogs (10 females; 5.9±2.6 y-old) and was fed a maintenance diet throughout the study. Serum metabolites were analyzed through ^{1}H Nuclear Magnetic Resonance spectra (NMR). Principal component analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) were performed using pareto scaling pre-processing to the data set (64 spectra × 236 columns) to assess qualitative differences in serum metabolomic profiles between control and CKD, and differences within the CKD group before (T0) and after (180 days; T180) renal diet exposure. **Results and discussion.** Compared to control dogs, CKD dogs (T0 and T180) showed an altered metabolic serum profile with increased levels of urea, creatinine, creatine, citrate, several lipids and lower levels of lactate, branched-chain amino acids (BCAA) and glutamine (Figure 1). Within the CKD group we noted that there was no difference on metabolite profile between T0 and T180. It is expected that six months of CKD would have altered the metabolic profile of the patients. However, our findings indicate that diet intervention may slow disease progression, which corroborates previous studies in dogs [5] and humans [3].

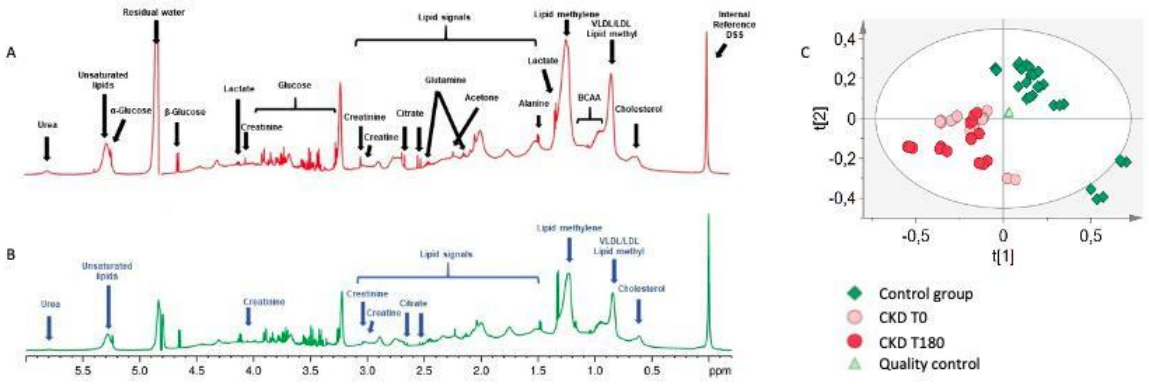

Figure 1. Expansion (-0.2 – 6.0ppm) of 1D NOESY-presat 1H NMR spectra (600 MHz, 300 K) of the healthy and CKD groups (**A)** shows all altered molecules of CKD group after 180 days of renal diet; (**B)** shows the group of healthy dogs (control diet), highlighting the metabolites with different profiles compared to the CKD group **(C)** Partial Least Squares Discriminant Analysis (PLS-DA) score scatter plot.

Conclusion. Metabolomics profile can be used to differentiate between healthy and sick animals. Renal diet may slow down progression of CKD.

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Hepatic intermediary metabolism of rainbow trout fed commercial amino acid balanced diets with increasing levels of a partially defatted Tenebrio molitor meal

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Introduction. Insect meals are interesting candidates to replace fishmeal (FM) in diets for cultured fish [1]. The inclusion of Tenebrio molitor meal (TM) in the diet of aquaculture species showed promising results in terms of fish health and growth performance [2]. In rainbow trout, the absence of chitinase activity could interfere with protein digestion and amino acid (AA) utilization regardless the AA balancing in diets. Likewise, dietary fatty acid (FA) composition could influence de novo FA synthesis by altering the activity of lipogenic enzymes. This study evaluated the activities of key hepatic AA catabolizing and lipogenic enzymes of grow-out rainbow trout fed a commercial diet, balanced in AA profile, containing increasing levels of a partially defatted TM in substitution of FM.

Animals, material and methods. 252 trout were randomly allotted to 4 isonitrogenous, isolipidic and isoenergetic diets control (T0; 20% FM) and 5 (T5), 10 (T10) and 20% (T20) TM inclusion (Table 1), with 3 tanks as replicates and 21 fish/tank. The TM-containing diets were supplemented with essential AA to provide comparable and balanced AA profile as T0. After 154 days of trial, liver samples were collected (9 replicates/treatment). The activities of AA catabolizing alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), glutamate dehydrogenase (GDH) and lipogenic glucose-6-phosphate dehydrogenase (G6PD), malic enzyme (ME), fatty acid synthetase (FAS) enzymes were measured [3]. Data were tested with One-Way ANOVA.

Results and discussion. The dietary treatment did not affect the activities of the considered enzymes (Table 2). Such response is attributable to: (i) comparable AA intake by the trout of the 4 experimental groups (for ALAT, ASAT and GDH) [4]; (ii) comparable EE, EPA, DPA and DHA contents in the experimental diets (for G6PD and FAS) [5]; and lack of growth rate variations among groups (for ME) [6].

Table 1. Chemical composition of the experimental diets.

Item	T0	T5	T10	T20
DM (%)	93.8	93.8	94.1	94.4
CP (%DM)	42.1	43.1	43.4	44.3
EE (%DM)	22.6	23.0	22.4	22.4
GE (MJ/kg)	22.2	22.7	22.7	22.6
EPA (g/kg DM)	10.2	10.6	10.4	10.5
DPA (g/kg DM)	3.8	4.1	3.5	3.2
DHA (g/kg DM)	1.0	1.4	1.6	1.0

DM, dry matter; CP, crude protein; EE, ether extract; GE, gross energy; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

Table 2. Enzyme activities (mU/mg protein) in trout fed the experimental diets.

Item	T0	T5	T10	T20	SEM	p
ALAT	312.7	326.3	359.0	361.4	9.37	ns
ASAT	635.0	487.0	522.4	634.3	22.66	ns
GDH	38.0	33.4	36.7	37.2	1.17	ns
G6PD	228.7	195.8	208.5	226.7	7.70	ns
ME	78.0	63.4	65.5	76.8	2.52	ns
FAS	4.5	5.3	3.8	4.5	0.23	ns

SGR, specific growth rate; SEM, standard error of the mean; ns, not significant.

Conclusion. In current commercial diets for trout, TM can totally substitute FM without any negative effects on protein digestion and hepatic AA catabolizing and lipogenic enzymes.

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Limits of an easy system to simulate in vitro gastric and small intestine digestion of horses

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Introduction: Animal welfare and the reduction of animal experiments become increasingly important in sciences. For this reason, usable systems for the simulation of digestion processes in animals have to be developed. In the present study the system to simulate gastric and small intestine digestion of horses was refined to reduce losses of water soluble substances. The hypothesis was that after evaporation of liquids only minor losses of nutrients occur.

Material and methods: In the present investigation apple pectin, maize starch and inulin were used. An amount of 20 g of each feedstuff was stirred in 38°C warm water. The pH-value was adjusted to 3.2 +/- 0.05 with 1M HCl-solution. In the next step 2.28 g/l Pepsin (pepsin 2000 FIP-U/g, EC 3.4.23.1, Merck KGaG, Darmstadt, Germany) was added and the mixture was incubated for one hour at 38°C under constant stirring. Thereafter, preheated phosphate buffer (4.4 g/l KH_2PO_4, 4.6 g/l Na_2HPO_4) was added and the pH-value was adjusted to 6.9 +/- 0.04 with 1M NaOH-solution. To simulate small intestine digestion 0.25 g/l Pancreatin was added and the mixture was incubated for one hour at 38°C under constant stirring[1]. The mixture was filtered and the solid rest dried by 60°C in the oven. The liquid phase was evaporated, when water-soluble substances were digested (pectin and inulin). In the digested material total water-soluble carbohydrates as well as fructan[2] and in case of maize starch, starch, were analysed.

Results and discussion: In dry matter (DM) the content of fructan in the pectin was 3.2%, whereas the residue in the filter after digestion contained 0.8±0.04% and the evaporated material contained 6.0±1.2%. For total soluble carbohydrates the amounts were 20.6%, 3.4±0.2% and 41.3±7.7%, respectively. The inulin contained originally 31.8% DM fructan and 56.4% DM soluble carbohydrates, from the digested material there remained no residues in the filter. In the evaporated liquid face were 26.6±1.6%/DM of fructan and 47.7±2.6% DM total soluble carbohydrates. After digestion of pure starch the filter residues as the undigested starch contained starch of nearly 100% of DM. As expected after digestion of pectin and inulin the liquid phase contained high amounts of water-soluble carbohydrates. However, evaporation did not seem to be the suitable method to save these substances for further digestion together with the filter residues in the fermentation system “Caesitec” to simulate the whole digestion process of horses or pigs.

Conclusion: In conclusion, the in vitro system after Strauch et al. (2017)[1] simulating gastric and small intestine digestion of horses has to be advanced to produce data on foregut digestion that reflect the in vivo processes. Only if water-soluble substances can be preserved in the digested material for the following fermentation, the in vitro simulation of horse digestion will be suitable.

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Serum metabolomics analysis reveals that weight loss in obese dogs results in similar metabolic profile than that of dogs in ideal body condition

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Introduction. In recent decades, the incidence of obesity in companion animals has increased significantly and become a serious concern in veterinary medicine [1]. The study of metabolic profile can be an important tool to better understand, at a systemic level, how metabolic alterations caused by different pathological conditions, such as obesity. Besides, it allows the discovery of metabolic biomarkers, which may help to diagnose alterations applied by obesity. However, there are few studies evaluating the hypothesis that obese dogs have changes in the metabolomic profile in relation to control animals; and that weight loss modulates these changes, returning to normality. **Animals, material and methods.** All procedures were previously approved by the Animal Use Ethics Committee (protocol number 4668091214). Ten obese spayed female dogs of owners, with body condition score (BCS) 9 [2] and aged 1 to 8 years were included and body composition was determined by the deuterium isotope dilution method. The dogs were subjected to a weight loss program [3] fed with restricted calories corresponding of 60% of their energy requirement for maintenance and have been reassessed after losing 20% of the initial body weight. A second experimental group was composed of ten lean spayed female dogs of owners in ideal BCS and aged 1 to 8 years (control group). All animals consumed the same diet for 28 days. Metabolites were identified on the high-resolution ^{1}H Nuclear Magnetic Resonance (NMR) spectra of the animals' blood serum [4]. **Results and discussion.** Twenty metabolites were identified; the metabolomic results differs between obese and dogs with ideal body condition score. Meantime, weight loss changed the metabolomic profile to a pattern similar to those observed in animals in ideal body condition. The metabolites that contributed to the separation of the groups were: glucose, lactate, glutamine, acetone, arginine, alanine, and citrate for the group of healthy animals (control and after weight loss); and lipids, cholesterol, and branched-chain amino acids for the obese animals. The discussion of the results obtained is very rich and allow a complex comparison between animals in ideal body condition, obese and after weight loss, in the most varied metabolic and physiological routes. For example, the greatest risk of developing urolithiasis in obese animals, according to the theory of inhibition of crystallization, can be justified by the lower concentrations of citrate verified by the metabolomic analysis in this group.

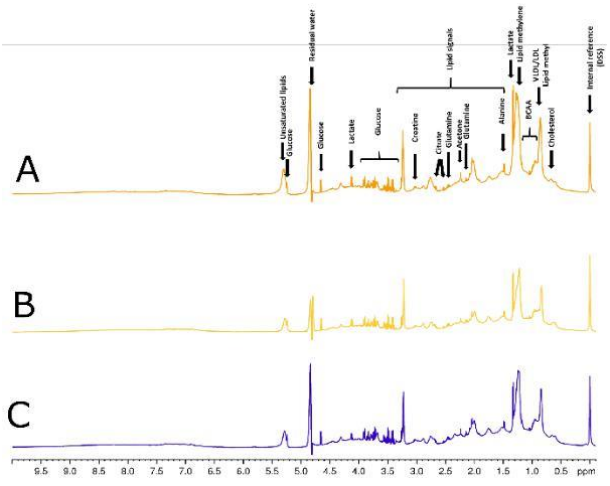

Figure 1. Representative attributed ^{1}H NMR NOESY-presat spectrum of obese (A), after weight loss diet (B) and control group (C). **Conclusion.** The metabolomic assessment based on nuclear magnetic resonance (NMR) of serum differs between obese dogs and animals in optimal body condition. Moreover, weight loss results in metabolomic profiles similar to those observed in lean animals. **References:** [1] German 2006 J. Nutr., 136: 1940-1946; [2] Laflamme, D. P. 1997 Canine Pract., 22(3): 10- 15. [3] Brunetto M. A. et al. 2011 Br. J. Nutr., 106: 194-197; [4] Beckonert O. et al. 2007 Nat. Protoc., 2: 2692-2703.

Investigation of gene expression with no sequence data: study on Reeves's muntjac (*Muntiacus reevesi*)

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Introduction: The real time PCR technique is a very popular method for gene expression analysis. Analysis of gene expression with this technique requires gene specific primers which are designed on the basis of sequences available in the GenBank collection. However, many genes are not yet sequenced, especially for species not commonly used in research. Thus, the aim of this study was to develop a method of designing primers for gene expression analysis (used in the nutritional study; for details see companion abstract; Przybyło et al.) using real-time PCR when there is no sequence data available in GeneBank for the particular animal, in this case Reeves's muntjac (*Muntiacus reevesi*).

Animals, materials and methods: The research project aimed to investigate the mRNA expression of the following: GAPDH (glyceraldehyde 3-phosphate dehydrogenase), ACTB (beta-actin), NaKATPase (sodium–potassium adenosine triphosphatase), SGLT1 (glucose transporter in rumen and small intestine), GLUT2 (glucose transporter in small intestine), GLUT5 (fructose transporter in small intestine), MCT1 (monocarboxylate transporter 1), GPR41 and GPR43 (G-protein coupled receptor 41 and 43, respectively) and T1R3 (taste receptor type 1 member 3). Having in mind that gene structure is similar between related species[1] we decided to put together all sequences for selected genes of species related to muntjacs which are available in GenBank. For selected genes we compared sequences for white-tailed deer (*Odocoileus virginianus*), cattle (*Bos taurus*), goat (*Capra hircus*) and sheep (*Ovis aries*). Additionally, when available, mRNA sequences of the following species were also considered: red deer (*Cervus elaphus*), bison (*Bison bison*), water buffalo (*Bubalus bubalis*), zebu (*Bos indicus*) and yak (*Bos mutus*). Subsequently, all sequences were aligned to identify highly conservative regions. For these regions we designed specific primers which served for the Real-time PCR analysis. The test material was obtained from four different parts of muntjac gastrointestinal tract: atrium ruminis, ventral sac of rumen, duodenum and jejunum. Samples were used for total RNA isolation and reverse transcription. Subsequently, cDNA samples from all four regions were mixed to create a pooled sample. Target and reference gene mRNA expression was determined using Real-time PCR. The product of each reaction was sequenced, both forward and reverse. Obtained sequences were used to search gene bank collection, and during alignment both query cover and percent of identity were considered.

Results and discussion: The query cover for analyzed PCR products was usually high (≥90%). Lower values (from 80% to 90%) observed for both, forward and reverse, products of sequencing for MCT1 and GPR41 might be due to the higher sequence variability of these genes between species. Considering the percent of identity, the majority of investigated products had high sequence homology (>90%) to the intended target among compared species. Only the product for GPR43 had lower sequence homology (average 85,4%), which might have resulted from the greater occurrence of single nucleotide polymorphism in the gene sequence.

Conclusion: The presented method of designing primers seems to be reliable and gives the opportunity to study expression of genes in the less known animal species. The study was supported by the Ministry of Science and Higher Education in Poland (DS-3217/KZiDZ/2018).

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Validation of reference genes for quantitative real-time PCR in gastrointestinal tract tissues of Reeves's muntjac (*Muntiacus reevesi*)

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Introduction. Gene expression studies require identification of reference genes with stable expression for accurate normalization. In most cases, preliminary selection of reference genes is based on literature data. However, such data may not be available when it comes to animals not commonly used in research, like Reeves's muntjacs. Hence, the purpose of the study was to select the most stable reference gene or combination of genes that could be used for normalization of gene expression data at mRNA level. Furthermore, suitability of investigated reference genes in nutritional study was verified.

Animals, materials and methods. Three reference genes most commonly used in the studies on ruminants: glyceraldehyde 3-phosphate dehydrogenase (GAPDH), beta-actin (ACTB) and sodium–potassium adenosine triphosphatase (NaKATPase), were chosen for validation. Samples from atrium, ventral sac of the rumen, duodenum and proximal jejunum were derived from eighteen males of Reeves's muntjac (*Muntiacus reevesi*) and served for total RNA isolation followed by reverse transcription. The animals were fed three diets (6 animals/treatment) differing in intake of mono- and disaccharides (for details see companion abstract; Przybyło et al.). Expression of the selected genes in cDNA samples was tested using quantitative real-time PCR. To exclude effect of treatment, the expression data was analyzed using the MIXED procedure of SAS as randomized complete block design. Estimation of gene expression stability was carried out separately for each part of the gastrointestinal tract using stable genes statistical software – NormFinder[1].

Results and discussion. Expression of all tested genes was unaffected by treatment ($P > 0.10$). Analysis of stability showed that expression of NaKATPase was most stable in all investigated parts of gastrointestinal tract. Best combination of two genes recommended by Normfinder was NaKATPase and ACTB for atrium, duodenum and proximal jejunum, and NaKATPase and GAPDH for ventral sac of the rumen.

Conclusion. On the basis of obtained results it can be concluded that the most stable gene for normalization of gene expression in the gastrointestinal tract of muntjacs is NaKATPase. However, since it is one of the first studies in this subject, and covers only results for three genes, suitability of other genes for normalization of gene expression data should be tested.

The study was supported by the Ministry of Science and Higher Education in Poland (DS-3217/KZiDZ/2018).

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The effect of dietary sugars on selected genes expression in the gastrointestinal tract of Reeves's muntjac (*Muntiacus reevesi*)

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Introduction. Increased intake of mono- and disaccharides (MD) by captive ruminants raises concerns, due to possible negative impact on the gastrointestinal tract (GIT). These concerns are raised by the often reported presence of fruits and vegetables (abundant sources of MD) in the diets for captive ruminants, which has been associated with some pathological changes in the GIT, low feed intake and poor body condition of animals. In several studies fruits and vegetables accounted even for 15% of DM fed to zoo ruminants, which had to result in high intake of MD (even over 15% of consumed DM). MD are rapidly fermented in the rumen, which may result in a rapid short-chain fatty acids (SCFA) increase in the digesta and the digesta pH drop below the optimum. However, due to faster digesta flow out of the rumen in browsing ruminants, some part of dietary MD may escape the rumen and affect lower regions of the GIT. Therefore, the dietary MD may affect functions of not only the rumen, but also the intestine, at least its proximal sections. The aim of this study was to determine the effect of addition of a MD mixture into the diet on SCFA and glucose transporters, and SCFA receptors in the GIT of Reeves's muntjac (Muntiacus reevesi), a browsing ruminant representative.
Animals, materials and methods. Eighteen male Reeves's muntjacs were fed diets consisting of dehydrated chopped lucerne (ad libitum), high-fiber pellet (100 g/day) and wheat bran (30 g/day) without (MD0) or with addition of 10 or 20 g of glucose, fructose and sucrose mixture/day (MD10 and MD20, respectively). Doses of mixture of MD were set to increase intake of these saccharides by 25 and 50% relative to MD0, which resulted in a range of water soluble carbohydrates content in consumed dry matter from 7.5 to 12.1%. Samples of epithelia from atrium and ventral sac of the rumen, and duodenum and proximal jejunum were collected after 14 days of adaptation to the experimental diets, two to three hours after feeding. The samples served for total RNA isolation followed by reverse transcription. Expression of SGLT1 (glucose transporter in rumen and small intestine), GLUT2 (glucose transporter in small intestine), GLUT5 (fructose transporter in small intestine), MCT1 (monocarboxylate transporter 1), GPR41 and GPR43 (free fatty acid receptor 3 and 2, respectively) at the mRNA level was determined using quantitative real-time PCR. Sodium–potassium adenosine triphosphatase (NaKATPase) was used as a reference gene. Results were analyzed using the MIXED procedure of SAS with data analyzed as randomized complete block design.
Results and discussion. Expression of SGLT1 in the duodenal epithelium was less for MD10 compared to MD0 and MD20, but did not differ between MD0 and MD20. No other differences between treatments were observed ($P > 0.05$).
Conclusion. The results of this study suggest that dietary sugars may affect SGLT1 expression in the duodenum of Reeves's muntjac and that this effect may be dose dependent.

Influence of starch gelatinisation on nutrient digestibility in laboratory mice

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Introduction: The degree of starch gelatinisation in a diet depends on feed processing technology. It has been shown that maintenance laboratory animal diets available in different confection (pelleted vs. extruded) are often marketed as identical, but differ in starch gelatinisation[1]. Starch gelatinisation influences carbohydrate digestibility and is an indirect way to estimate the degree of thermal processing of the diet. However, no information regarding starch characteristics is given on diet labels or in publications using such diets. Therefore, this study aimed to test the hypothesis that the difference in starch gelatinisation has an impact on nutrient digestibility in laboratory mice.

Animals, materials & methods: Eight-week-old C57Bl/6 mice were used for the tri-al. They were fed a commercial, fortified lab animal maintenance diet in pelleted or extruded form (groups P and E, each n=11). Starch content was 26.6% and 27.1%, the degree of starch gelatinization (measured according to VDLUFA standard meth-od III 7.2.6) was 15.2% and 56.7% in diet P and E, respectively. After one week of adaptation to the respective diets, the total amount of faeces per cage (2-3 mice) was collected for 15 days. Diets and faeces were analysed for macronutrients to calculate the apparent digestibility (aD) of gross energy (GE), crude protein (CP), ether ex-tracts (EE), the carbohydrate/fibre fraction (N-free extracts + crude fibre; CH+F) and calcium (Ca) and phosphorus (P). Groups were compared with the Student's *t*-test (SigmaPlot software; significance level: $p<0.05$).

Results & Discussion: Group E showed significantly higher aD of dry and organic matter, GE, CH+F, Ca, and P (Table 1), while there was no significant difference in aD(CP) and aD(EE).

Table 1: Apparent digestibility of dry matter and nutrients (%; means ± SD; *t*-test; $p<0.05$).

	group P	group E	*p*
Dry matter	70.8 ± 0.8	77.2 ± 0.7	<0.001
Organic matter	73.7 ± 0.6	80.3 ± 0.5	<0.001
GE	74.7 ± 0.5	80.9 ± 0.6	<0.001
CH+F	69.17 ± 0.71	79.05 ± 0.45	<0.001
CP	82.2 ± 0.6	81.7 ± 0.9	0.306
EE	91.2 ± 0.7	92.0 ± 0.5	0.094
Ca	14.5 ± 4.6	27.7 ± 7.9	<0.05
P	26.4 ± 3.9	41.8 ± 7.4	<0.01

The results confirm the hypothesis that the extrusion process and therefore, as a ma-jor factor, the higher starch gelatinisation degree leads to a better digestibility of diet E. This may have an impact on gastrointestinal as well as intermediary metabolism and it may affect the percentage of metabolisable energy from carbohydrates. In terms of standardisation of laboratory animal research, P and E diets are not compa-rable.

Conclusion: Commercial diets for laboratory animals should be labelled with information on degree of starch gelatinisation. This information should be provided in pub-lications about animal trials.

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Impact of two different diets on faecal parameters of horses

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Introduction. Forages represent the dietary basis of horses, while high amounts of starch can impact their intestinal health [1]. Since few studies are conducted on the faecal parameters (faecal quality and proximate analysis through near-infrared reflectance spectroscopy - NIR), this study aimed to evaluate differences in faecal parameters among horses either fed with a forage-based diet or fed with high amounts of concentrates.

Animals, material and methods. Samples of fresh faeces were collected after defaecation from 18 healthy horses (mean (±SD) age 4.78±1.17). 9 horses were fed hay ad libitum (hay group-HG). 9 horses were fed hay and 8 kg/animal/day of a cereals pellet (hay+concentrate group-HCG) supplied in 2 meals/day. Hay used was first-cut meadow with green colour. Concentrate was based on corn and barley feed (crude protein 14.50%, ether extract 3.50%, crude fibre 5.70%, ash 6.60%). Faeces were scored for faecal colour (FCl) and faecal consistency (FCn). FCl was scored through a scale developed by the authors (brown, grey, brown/yellow, brown/green, green). FCn was scored as described by Berg et al. [2]. Faecal hardness (FH) was measured in 50 g fresh faeces with a fruit penetrometer (GY2, Beslands). Faecal pH (FpH) was measured with an electronic pH meter (precision±0.01; Hach-Lange). FH and FpH were measured in triplicate. Fresh faeces (150 g) were dried in a forced ventilation oven (55°C for 72 hours). After drying, samples were grinded (1-mm sieve) for determining faecal components (neutral detergent fiber-NDF, acid detergent fiber-ADF, acid detergent lignin-ADL, crude protein-CP, starch, and ash) through NIR. Statistical analysis was performed (SPSS 21.0). Data were compared by Student t-test ($P<0.05$). Frequency of scores (%) for FCl and FCn was assessed by using Chi-square test.

Results and discussion. Faecal parameters varied between the diets (Table 1). There were effects of the diet on FCl and FCn ($P=0.001$): samples were scored brown/green with 3/5-points score for FCn in HG; and grey with 5/5-points score for FCn in HCG.

Table 1. Faecal parameters in HG and HCG. Data are expressed as mean±SD

	DM %	FpH	FH kg/cm²	NDF %DM	ADF %DM	ADL %DM	CP %DM	Ash %DM	Starch %DM
HG	26.40 ± 1.17	6.77 ± 0.21	3.55 ± 0.80	75.85 ± 0.67	55.30 ± 0.33	20.99 ± 0.62	6.87 ± 0.42	10.67 ± 0.42	0.15 ± 0.82
HCG	17.74 ± 1.97	6.01 ± 0.34	2.07 ± 0.64	65.68 ± 1.81	45.42 ± 1.17	15.60 ± 0.96	10.73 ± 0.42	10.95 ± 0.28	2.00 ± 0.37
P	<.01*	<.01*	<.01*	<.01*	<.01*	<.01*	<.01*	0.58	<.01*

Conclusion. Diet has an impact on DM, faecal consistency, pH or faecal components. In HG, brown/green colour of faeces and harder consistency reflect the impact of hay intake on the faeces quality and intestinal health [3]. Faecal pH of 6.77 in HG reflects a typical fiber fermentation profile in the large intestine. The lower faecal pH in HCG seemed to be related to a higher starch fermentation profile with lactate production in the hindgut. This work highlights the evaluation of faecal parameters as useful tool to monitor the effects of diet on intestinal health.

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RETROSPECTIVE STUDY OF THE COMPLICATIONS IN THE USE OF NASOESOPHAGEAL OR NASOGASTRIC FEEDING TUBES IN DOGS

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Introduction: Hospitalizing dogs for feeding can be expensive and inaccessible for some owners. In our routine, we use various enteral feeding tubes to support animals at their homes. This study aimed to compare the complications of nasoesophageal (NE) or nasogastric (NG) tubes in dogs that received this support in their homes.

Animals, materials and methods: Retrospective data of dogs submitted to tube feeding from December 2013 to January 2015, at the Nutritional Service of Teaching Veterinary Hospital were separated into two groups: NE, nasoesophageal tube at the 10th intercostal space; NG, tube in the stomach. Only dogs with tube positions verified by X-ray fed for at least 3 days and owners that followed given feeding instructions were included. Variables assessed (at the tube placement) were: body weight (BW), body condition score (BCS), muscle mass score (MMS), days of hyporexia/anorexia, age, and tube diameter. The days with the tube (DT), energy supplied, complications (vomit, diarrhea, sneeze, cough, epistaxis, clogging of the tubes), and changes in body weight (±5%) were assessed at the last day of tube feeding. Variables were compared between groups with Chi-square or Mann-Whitney U test ($P<0.05$).

Results and discussion: Data from 172 dogs were collected. From these, 100 (39 NE and 61 NG) used the tube for more than 3 days: mixed breed (n=57), Poodle (n=10), Shih-tzu (n=7) and other breeds (n=26). Multisystemic diseases (n=34) were the most frequent reason for hyporexia/anorexia, followed by gastrointestinal (n=23), genito-urinary (n=20) and others (n=23). No differences between tube types was verified ($P>0.05$), and for the NE group the median age was 8.0 (0.2-14.0) years; BW 7.5 (1.0-45.6) kg; BCS 4.5 (1-8); MMS 2.0 (0-3); median of hyporexia/anorexia 3.5 (0-30) days; tube diameter 8 (6-14) french; and the DT 6.0 (3-20) days. On the NG group the median age was 7.0 (0.2-16.0) years; BW 9.8 (2.3-41.7) kg; BCS 3.9 (1-8); MMS 2.0 (0-3); median of hyporexia/anorexia 4 (1-30) days; tube diameter 10 (6-14) french; and DT 5 (3-28) days. Other parameters assessed are on Table 1. Our results are similar to previous study that included smaller number of dogs [1].

Conclusion: Placing feeding tube end in the esophagus or in the stomach does not change complication rate and outcome of dogs in hyporexia/anorexia.

Acknowledgements: Affinity Pet Care for financial support.

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Table 1. Variable evaluated according to tube feeding placement, in each group.

Variables	NE	NG	P value
Mean energy supplied [kcal/kg$^{0.75}$/d (range)]	80 (40-95)	79 (11-128)	0.32
	% of dogs in each group		
Body weight lost	36.3	37.2	1.00
Body weight maintained	42.4	41.8	1.00
Body weight gain	21.3	21.0	1.00
Gastrointestinal complications	40.6	40.0	>0.63‡
Respiratory complications	60.2	52.4	>0.15¥
Clogging of the tubes	5.1	6.6	1.00
Death	26.4	27.6	1.00

NE = Nasoesophageal group. NG = nasogastric group. ‡Vomit (p=0.8); diarrhea (0.6); vomit and diarrhea (1.0); none (1.0). ¥Sneeze (0.5); cough (0.3); epistaxis (1.0); none (0.1).

Feeding Management and Health of horses in Arid Climates

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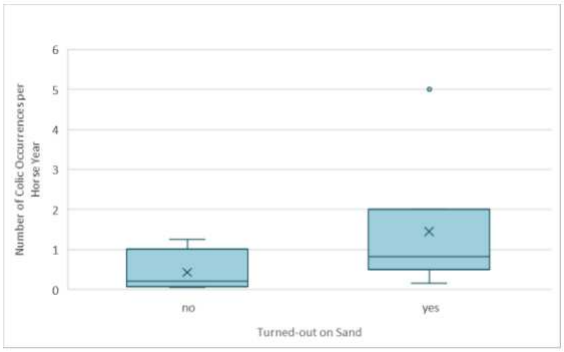
Introduction. Current equine nutrition and management research as well as recommendations focus mostly on temperate humid climates. However, the middle eastern arid climate provides different challenges in horse management and nutrition. In addition, due to climate change, global desertification may increase [1]. Israeli horse management relies on low-quality legume and cereal hay [2] barley-grains, and limited access to grass, while many horses are turned out on sand. We aimed to survey feeding practices and the occurrence of colic across Israel in relation to management. **Methods.** Horse owners completed an online survey for 465 horses, distributed across 4 climatically distinct areas of Israel. Data were analysed using JASP (version 0.11.1, 2019; sig.p<0.05). Data were analysed using non-parametric tests (X^2, Mann Whitney and Spearman Rank Correlation). Colic means per horse year (/HY)± standard error are reported, unless otherwise specified, as reported by horse owners.

Results and Discussion. The mean colic occurrence was 0.11±0.01/HY for all horses and 0.45±0.03/HY within reported colic horses (n=112), while 29 respondents provided a possible diagnosis. Of these, 50% were reported as impaction, 14% as impaction and dehydration and 11% as dehydration. Sand accumulation was mentioned for only 14%, although 57% of these horses and 48% of all horses were turned out on sand. Horses who had access to sand recorded a significantly higher occurrence of colic (0.2±0.01/HY) compared to others (0.07±0.02/HY) (Figure 1.a, p=0.002, Mann-Whitney). The risk for colic increased 1.9 times when horses were stabled on sand and increased 1.4 times on sandy pasture. Horses with reported gastric ulcers had a greater occurrence of colic (p<0.001, Mann-Whitney). Surgery was reported in 13% of the colic horses. Feeding low-quality oat and alfalfa hay (late cut, stalky, dusty) increased risk of colic by 1.5 compared to other feed stuff (Figure 1.b, p=0.03, χ^2).

a)



b)

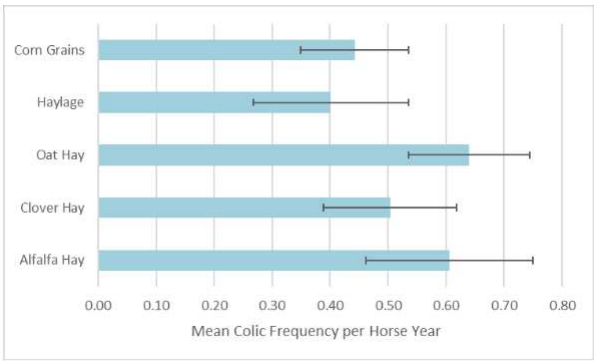


Figure 1. a) Colic risk as a factor of turn-out on sand. (p<0.05) **b)** Colic risk as a factor of different feed types (p=0.03) (±s.d.)

Conclusion. Preliminary results confirm similar risks for colic to previous studies which may be exacerbated by feeding management in arid climates.

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Leukoencephalomyelopathy in cats

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Introduction.

Several case series of acquired leukoencephalomyelopathy have been described in specific-pathogen-free cats that were fed irradiated cat food. We hypothesized that abnormalities in fatty acid metabolism in myelin might play an essential role in the pathogenesis of this acquired form of leukoencephalomyelopathy in cats. Therefore, we investigated the fatty acid profile of the white matter of the spinal cord of affected cats and control animals that were presented at Utrecht University between 1989 and 2001, and of irradiated and non-irradiated food that has been fed to these cats.

Animals, material and methods.

The affected cats developed progressive paresis/ paralysis and ataxia. These cats otherwise had normal appetite, water consumption, reproduction, and behavior. The three control cats, which were used for fatty acid determinations of the spinal cord white matter, were aged-matched cats (between 2 months and 2 years of age) that died of non-neurological diseases. Spinal white matter was collected from nine affected cats that were euthanized because of severe neurological symptoms, (three affected cats from the 1997 outbreak and six affected cats from the outbreak in 2001), as well as from the three control cats. Also a sample of the irradiated food associated with the 2000 outbreak (SSNIFF, 2.5Mrad = 25kGy) and of a standard dry cat food from the same manufacturer were obtained and ground prior to analysis. The only difference between these diets was the irradiation. Fat content of the diets was 10.0% on dry matter basis. Fatty acid analysis was done by HPLC/MS. As this was not a pre-determined study and the animals died or were euthanized because of humane endpoints before enrollment, no ethical approval was needed. Data were not statistically analyzed.

Results and discussion.

The irradiated food had low levels of alpha linolenic acid (1.38% of total fatty acids, compared to 2.90% before irradiation) compared to linoleic acid (21.85% versus 26.30%) as well as a high total omega-6:omega-3 ratio of 7:1, compared to a ratio of 2:1 in the non-irradiated food. The white matter of the spinal cord showed low levels of linoleic acid (1.52% versus 4.57%) and absence of alpha linolenic acid in affected cats (0.00% versus 0.25%) as well as absence of lignoceric and nervonic acid in both affected and control cats.

Conclusion.

These discrepancies in fatty acid composition of the white matter of the spinal cord may reflect an increased need for alpha linolenic acid as a substrate for longer chain omega-3 fatty acids to form myelin and thus indicate a particular species sensitivity to dietary deficiency in omega-3 polyunsaturated fatty acids, particularly alpha linolenic acid in cats. Our findings indicate that abnormalities in fatty acid metabolism in myelin play an essential role in the pathogenesis of this acquired form of leukoencephalomyelopathy in cats.

References: This study has been published in the Journal of Animal Physiology and Animal Nutrition. Leukoencephalomyelopathy in cats linked to abnormal fatty acid composition of the white matter of the spinal cord and of irradiated dry cat food. Ingh, T.S.G.A.M. van den, Grinwis, G.C.M., Corbee, R.J. Journal of Animal Physiology and Animal Nutrition, 2019, 103, 1556–1563.

Session 4B Protein

Chair: Stefanie Handl

Mechanically deboned chicken meat as protein source for extruded diets for cats: digestibility, palatability, and fermentation products on feces

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Introduction: Protein sources are key ingredients to reach the nutritional quality and marketing claims of cat foods. Traditionally, poultry or chicken by-product meal (CM) is the most relevant animal-derived protein in pet food and has low moisture content. However, new extrusion technologies make possible the inclusion of high levels of moisture raw materials, such as fresh (frozen) meat. The present study compared the replacement of CM by mechanically deboned chicken meat (MDCM) as a protein source for extruded cat foods.

Animals, materials, and methods: A grain-free formulation was balanced for cats, and the CM replaced resulting in four diets (inclusion on the as-fed basis of the raw material mixture): control (0%MDCM, only CM as a protein source), 40%MDCM, 80%MDCM and 120%MDCM (120kg of MDCM addition for each 100kg of dry raw material mixture inclusion). All diets presented similar contents of protein, fat, starch, and energy. Due to the high moisture content of the MDCM (79%), the substitution rate of the dietary protein was 0%, 17.4%, 31.4%, and 42.4%, respectively. Diets were produced at Wenger Extrusion Center (Sabetha, KS, EUA), and the high inclusion of meat-based raw material as possible using a specially designed twin-screw extruder (Thermal Twin, Wenger, EUA). Twenty-four mixed breed cats (3.5±1.5 y; 4.0 ± 0.5 kg) were used for the digestibility study (10 days adaptation and 7 days total collection of feces and urine, cats individually housed in cages), nitrogen balance, fermentation product on feces, and urine characteristics evaluations (Approval 012152/18). The palatability of the control diet (0%MDCM) was compared with the 40%MDCM, 80%MDCM, and 120%MDCM diets using the two-pan method with 40 cats. Data were submitted to analysis of variance and polynomial contrasts ($P<0.05$).

Results and discussion: Nutrient intake did not differ ($P>0.05$) among diets. The total tract apparent digestibility of DM, OM, CP, Starch, and fat were similar regarding MDCM inclusion ($P>0.05$). Feces characteristics and concentrations of short-chain fatty acids, ammonia, and lactate did not differ ($P>0.05$). Branched-chain fatty acids (BCFA) decreased linearly on feces with the inclusion of MDCM ($P<0.05$). Nitrogen balance and urine characteristics (volume, density, and pH) did not differ among treatments ($P>0.05$). Palatability comparisons (0%MDCM = 0.35 *versus* 40%MDCM = 0.65 intake ratio, $P<0.05$; 0%MDCM = 0.25 *versus* 80%MDCM = 0.75, $P<0.01$; 0%MDCM = 0.11 *versus* 120%MDCM = 0.89, $P<0.01$), and first choice (>90% for MDCM in all comparisons; $P<0.01$) indicate preference of MDCM formulations.

Conclusion: The substitution of CM by MDMC increased diet palatability with no influence on nutrient digestibility, nitrogen balance, and urine characteristics. Products of protein fermentation (BCFA) decreased on feces. The present extrusion system was able to produce appropriate formulations with high inclusion of raw materials with high moisture content.

Acknowledgments: Affinity Petcare Brazil and Manfrim Industrial Ltda (for the financial support to Laboratory); CNPq (fellowship for the first author).

Forty month-follow up of renal function in dogs fed a high-protein diet

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Introduction. Despite the absence of evidence of any deleterious effect of high-protein diets on pets' renal function [1-4], there are still concerns regarding the renal safety of such diets in the long term. Besides blood analyses commonly performed in vet practices, symmetric dimethylarginine (SDMA) has been shown to be an accurate kidney biomarker, not affected by dietary protein intake or lean body mass [5-7]. Our hypothesis was that a high-protein content in a complete and balanced diet had no impact on the renal function of healthy dogs. The objective of this study was to follow up selected renal parameters in adult dogs fed a dry high-protein diet for 40 months.

Animals, material and methods. Ten healthy entire adult Beagle dogs (16±6 month old), previously fed a standard maintenance diet, were fed exclusively a new high-protein diet (Table 1). Both diets met FEDIAF requirements for adult dogs [8]. The daily rations were calculated to maintain dogs' body weight. Fasting blood samples were collected at the start of the study (M0) and then every 2-3 months for 40 months, meaning 19 time points. Six serum parameters were measured to assess the renal function. Statistical comparisons were performed between each time point and M0 for each renal parameter, with 5% significance.

Table 1. Nutritional characteristics of the previous and test diets.

Nutritional characteristics	Previous diet	Test diet
Metabolisable Energy (ME) (MJ/kg as fed)	16.7	15.4
Protein (% ME)	25	34
Fat (% ME)	41	41
Carbohydrate (% ME)	34	25
Phosphorus (g/MJ)	0.60	0.64

Results and discussion. Individual values remained within the reference ranges over the study, except for urea in 1 dog (0.14 g/L at M29), creatinine in 1 dog (4.9 mg/L at M26), total protein in 3 dogs (50-51 g/L at M3, M29 and M33), albumin in 2 dogs (42-46 g/L at M9 and M15), phosphates in 1 dog (76.6 mg/L at M35), and SDMA in 5 dogs (15 µg/dL at M15, M22, M29, M37 and M40). These exceptions, very close to the reference values and without any correlation between them, can be considered incidental and due to biological variability of the biomarkers. Mean values of some criteria showed significant changes at some time points *vs* M0 (decrease for urea, creatinine, albumin and phosphates, increase for total proteins, and decrease or increase for SDMA), but always remaining in the safety ranges (Table 2).

Table 2. Mean values and standard deviations after 11, 24 and 40 months feeding the test diet (*: significant difference compared to M0).

Serum renal parameters	M0	M11	M24	M40	Range in study	Laboratory ref ranges
Urea (g/L)	0.32±0.10	0.29±0.05	0.28±0.05	0.23±0.05*	0.21-0.32	0.15 – 0.57
Creatinine (mg/L)	7.5±1.0	7.6±0.6	7.4±1.0	6.4±0.7*	6.4-7.6	5.0 – 18.0
Total proteins (g/L)	52.7±1.5	60.1±3.1*	58.4±2.6*	57.9±2.9*	52.7-62.7	52 – 82
Albumin (g/L)	36.2±2.6	34.3±2.8*	31.5±2.8*	31.6±2.5*	30.9-36.7	23 – 40
Phosphates (mg/L)	46.7±7.5	39.9±5.0*	41.6±4.4	38.3±2.7*	34.6-46.7	25 – 68
SDMA (µg/dL)	10.9±1.1	11.6±1.4	9.4±1.8	11.6±1.9	8.6-13.1	0-14

Conclusion. Our results showed that a high-protein content in a balanced diet had no negative impact on the renal biomarkers in healthy adult dogs during 40 months.

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Digestibility and palatability of extruded diets with mechanically deboned chicken meat for dogs

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Introduction: Poultry or chicken by-product meal (CM) is the most traditional protein source for extruded diets for dogs, an ingredient with low moisture after the rendering processing. New extrusion technologies have made possible elevated inclusions of high moisture raw materials, such as fresh (frozen) meat. The present study compared the replacement of CM by mechanically deboned chicken meat (MDCM) as a protein source for extruded foods for dogs.

Animals, materials, and methods: A formulation was balanced for dogs, and the CM replaced resulting in four diets: control (0%MDCM, only CM as a protein source), 40%MDCM, 80%MDCM and 120%MDCM (for each 100kg of dry raw material mixture inclusion, the additions into extruder preconditioner of 40kg, 80kg, and 120kg of MDCM, on the as-is basis). Final diets had similar contents of moisture, CP (except 120%MDCM, that presented unintentionally a little less protein), fat, and starch. The substitution rate of the dietary protein was 0%, 23.1%, 40.8%, 55.2%, respectively. The high inclusion of meat-based raw material as possible using a specially designed twin-screw extruder (Thermal Twin, Wenger, Sabetha, EUA). Twenty-four beagle dogs (4.0±0.8 years; 12.3 ± 1.5 kg), 6 per food, were used for the apparent digestibility study (5 days adaptation, 5 days total collection of feces and urine), nitrogen balance, fermentation product on feces, and postprandial urea response evaluations (Approval 012152/18). Palatability was compared by the two-pan test with 40 dogs (Panellis, Descalvado, Brazil). Data were submitted to analysis of variance and polynomial contrasts.

Results and discussion: Palatability comparisons (0%MDCM = 0.37 *versus* 40%MDCM = 0.63 intake ratio, $P<0.01$; 0%MDCM = 0.11 *versus* 80%MDCM = 0.89, $P<0.01$; 0%MDCM = 0.12 *versus* 120%MDCM = 0.88, $P<0.01$) indicate preference for MDCM formulations. DM, fat, and starch intake did not differ ($P>0.05$), but CP intake was lower for 120%MDCM ($P<0.05$) due to its lower CP content. Apparent digestibility of DM increased quadratically, and of the CP and gross energy increased linearly as the MDCM inclusion increased ($P<0.05$). By the polynomial regression, it was estimated that a diet formulated with only MDCM as a protein source would present 91.4% of CP digestibility ($R^2=0.44$; $P<0.05$). Feces characteristics did not change ($P>0.05$), but isobutyrate and ammonia concentration reduced quadratically, and lactate increased linearly as MDCM inclusion increased ($P<0.05$). A tendency for a total branched-chain fatty acid reduction was also observed ($P=0.06$), and altogether the fermentation products alteration suggests reduced protein fermentation after an increase in MDCM on diets. Plasma urea concentration and nitrogen retention did not differ between treatments ($P>0.05$).

Conclusion: The replacement of CM by MDCM increased diet palatability and the apparent digestibility of CP, DM, and gross energy, and reduced protein fermentation by-products on feces. The extrusion system evaluated was able to adequately process high moisture formulations that present MDCM as a first raw.

Acknowledgments: Affinity Petcare Brazil and Manfrim Industrial Ltda (for the financial support to Laboratory); CNPq (fellowship for the first author).

Precision feeding: how rumen protected amino acids can reduce the protein content of Parmigiano Reggiano rations?

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Introduction. Dietary protein content (CP) reduction is important to restrain nitrogen excretion and improve the nutrition efficiency also for cows producing milk for Parmigiano Reggiano (PR) cheese. In this production chain rations are based on local dry forages and cereals while silages, many protein sources, added fat and fermented by products are not allowed. The use of rumen-protected amino acids (RPA) helps in rations balance, reducing CP without compromising productions [1]. More knowledge about the optimal combination of carbohydrates sources (CHO), CP and RPA use is still needed for diet without silages. Aim of this study was to evaluate the effect of diets with high or low CP (HP *vs* LP) and a high or low starch (ST) content (HS *vs* LS). The main dietary CP were alfalfa hay and soybean meal. All diets were balanced using RPA (Smartamine® and AjiPro-L®) to have the same theoretical quantity of intestinal available methionine (M=71g/d) and lysine (L=191g/d.), as predicted by the NDS software (CNCPS 6.55).

Animals, materials and methods. 8 Italian Friesian cows were assigned to a Latin square design (14d adaptation, 4d sampling) with different diet profiles (%DM): HPHS:14.8 CP, 26.8 ST; HPLS 14.8 CP, 19.7 ST; LPHS: 12.5 CP, 25.9 ST and LPLS: 12.5 CP, 19.8 ST. Dry matter intake (DMI), daily water intake (DWI), body weight (BW), body condition score (BCS), rumination time (RT), reticular pH (r-pH) and milk yield (MY) were recorded. Ammonia in rumen fluid (ARF), milk and feces composition were determined. Energy corrected milk (ECM) was calculated [2]. Mixed model procedure was used for data analysis; period, CP and ST levels (HP *vs* LP and HS *vs* LS diets) and interactions were used as fixed effects, while animals as random effect. Multiple comparisons were then performed by Student's t-test.

Results and discussion. No differences in DMI (26.5kg/d, P=.22) and BW (636kg, P=.92) were observed, however higher BCS resulted in LP diets (+0.16pts, P<.05), suggesting a different energy utilization. DWI was higher in HP diets (+22 L/d; P=.05). RT was affected by CHO (-30min/d in HS, P=.01) but all diets induced good RT (505min/d) and r-pH resulted always safe excluding any risk of SARA. MY and ECM were higher with HP diets (+1.3, +1.5kg/d respectively, P<.01). Highest milk protein % resulted in HPHS diet (P<,05). ARF and urea content were influenced by HP diets (+1.3 ARF; +9.2 urea, mg/dl; P<.01). LP diets resulted in higher fecal starch concentration (+0.4, %DM, P<.01), showing a possible influence of diet CP on starch utilization in rumen and gut.

Conclusion. The results showed that diets with lower CP seemed inadequate to support MY and milk components even if balanced with RPA use. Moreover, the CP level of the ration to support the highest performances in the PR diet need to be higher than 12.5 (%DM) when only M and L are considered as potential limiting amino acids.

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Effects of dietary rye and rapeseed on performance, apparent CP digestibility and digesta characteristics in weaned piglets

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Introduction. The use of rye (RY) and rapeseed meal (RM) as feed ingredients in pig nutrition might have advantages over wheat (WH) and soybean meal (SM) based diets. RY is highly capable of coping with periods of drought [1] and has a unique fibre composition (high concentration of arabinoxylans) [2]. The use of RM as a protein source might reduce the import of soybean. Studies showed that the feeding of increasing levels of RY instead of WH had no negative impact on performance parameters of fattening pigs [3]. However, the feeding of RY might result in a high viscosity of digesta (V) and a reduced digestibility of crude protein (CP) in fattening pigs [4]. This study investigated the effect of feeding RY or WH combined with either RM or SM on performance, CP digestibility and digesta characteristics in weaned piglets.

Animals, material and methods. In total, 88 weaned piglets aged 28 days were allocated to 44 pens and fed four different experimental diets being isonitrogenous (n=11). Experimental diets were based on either WH/SM, WH/RM, RY/SM or RY/RM. Dietary inclusion levels were 48% for RY and WH, 30% for RM and 25% for SM respectively. During the feeding trial, body weight (BW) and feed intake were recorded weekly. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. After five weeks of feeding, one piglet per pen was euthanised. Digesta samples of jejunum and colon were taken and analysed for V and dry matter content (DM). Apparent ileal digestibility (AID) of CP was determined. Statistics were carried out using a 2-factorial ANOVA regarding the factors protein (P) and carbohydrate source (CHO) ($p<0.05$).

Results and discussion. The performance was influenced only by P, not by CHO. ADG and ADFI were higher for SM fed piglets compared to those fed RM between day 28-33 ($p<0.05$) and for the whole trial (day 0-33, $p<0.05$). ADFI was also lower for RM groups for day 0-7 and day 14 -21 ($p<0.05$). BW and FCR did not show any differences regarding the used P or CHO. Unexpectedly, the feeding of RY instead of WH resulted in a lower DM of digesta in jejunum and colon ($p<0.05$). V in the jejunum was higher for pigs fed RY ($p<0.05$). Precaecal AID of CP was influenced by P and CHO. It was higher in groups fed diets containing SM or WH ($p<0.01$). Thus, higher values of digesta V in RY fed piglets might lead to a lower AID of CP for both SM and RM.

Conclusion. The results of this study showed that the use of RY instead of WH had no negative impact on animal performance. Interestingly, CP digestibility was reduced in RY fed piglets, which might be related to changes in intestinal viscosity. SM as a protein source seemed to be superior to RM, resulting in better growth rates and digestibilities. Further investigations regarding effects on digestive function and gut health are needed in order to evaluate the pros and cons of rye and rapeseed as feed components in piglet nutrition.

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Effects of dietary choline supplementation on body weight, body condition, body composition, and lipid profile in post-gonadectomy kittens

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Introduction. Gonadectomy is considered a major risk factor for feline obesity [1]. Choline is known for its lipotropic effects in other species [2]. This study investigated whether lipotropic effects of choline supplementation on post-gonadectomy kittens could alter lipid metabolism by increasing fat mobilization for energy utilization.

Animals, Materials & Methods. This study was approved by the University of Guelph Animal Care Committee (AUP#4118). 15 intact male kittens (3-months old) were fed to growth requirements [3] using an extruded dry food base diet formulated for growth (3,310 mg choline/kg DM) during an 11-week acclimation [4]. Kittens were assigned to either base diet (n=7) or base diet with additional choline (n=8) (300 mg/kg $BW^{0.75}$). Post-gonadectomy (week 1), kittens were fed the base diet at 3 times their daily intake over 3 meals to mimic *ad libitum* feeding. Dual energy x-ray absorptiometry assessed body composition for fat mass gain (FMG), lean mass gain (LMG), and bone mineral content gain (BMCG) from week 0 to 12. Fasted serum samples were analyzed for triglycerides (TG), non-esterified fatty acids (NEFA), glucose, cholesterol (CHOL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL) [VLDL=TG/2.2] [5] and low-density lipoprotein (LDL) [LDL=CHOL-HDL-VLDL][5] were calculated. Daily food intake (FI), weekly body weight (BW) and body condition scores (BCS) were measured. Data was analyzed on SAS University using the proc mixed procedure with time and treatment as fixed effects and cat as subject. Statistical significance was set at $p<0.05$. **Results and Discussion.** Cats receiving choline had lower BW, BCS, and FI than base diet ($p<0.05$). FMG was lower with choline ($p=0.0051$) but no changes between groups were found for LMG or BMCG ($p>0.05$). No differences were detected ($p>0.05$) in serum TG, NEFA, CHOL, HDL, VLDL, LDL, or glucose between groups.

Table 1. Mean FI, FMG, LMG, and BMCG in base diet (n=7) compared to choline (n=8) groups

Variable	Base Diet	Choline	P Value
FI (g)	94.31±3.42	83.01±4.53	0.0001*
FM (g)	795.05±47.09	484.71±75.68	0.0051*
LM (g)	526.81±70.11	446.34±69.76	0.4325
BMC (g)	41.97±2.78	27.47±7.34	0.1040

Values expressed as mean ± SEM; *p<0.05

Conclusion. These results suggest a potential benefit of choline supplementation in post-gonadectomy kittens on FMG, BW, and FI during growth. However, these effects may not be attributable to changes in lipid mobilization. Future work should investigate further into the effects of choline on lipid metabolism in cats.

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Suitability of blood by-products for use as a sustainable protein source in diets for rainbow trout depends on processing method.

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Introduction.

Fish meal is considered an essential ingredient in feed for farmed carnivorous fish. As demands outgrew availability of fish meal produced from bycatch and trimmings, forage fish is purpose-caught to fill the gap. This led to rising feed costs and growing concerns on environmental sustainability of cultured fish, driving the industry to search for sustainable, cost-effective alternative protein sources[1]. For this purpose, low-ash animal by-products may be suitable ingredients for partial replacement of fish meal in aquafeed. We here evaluate the effect of processing method of porcine blood by-products on protein digestibility in trout: spray drying versus contact drying.

Animals, materials and methods.

The pepsin-HCl method[2] was used to determine *in vitro* protein digestibility of spray-dried hemoglobin (Actipro® 95PHS) and contact-dried blood meal (BM). Next, a digestibility trial was performed using 180 rainbow trout (148 8 g) in twelve 250 L-tanks with 15 fish each. Three pelleted diets were prepared: reference diet (RD) with 40% fish meal, 95PHS-diet (70% RD and 30% 95PHS) and BM-diet (70% RD and 30% BM). Fish were fed one of three test diets with 4 replicate tanks per diet. A 2-wk adaptation period and 2-wk feaces collection period were applied using a continuous automatic device and apparent protein digestibility coefficients (aD_CP) of test diets and test compounds calculated[3]. Statistics were done using one-way ANOVA and Tukey post hoc test with significance set at $p<0.05$.

Results and discussion.

The *in vivo* trial revealed an almost double digestible protein content in spray dried hemoglobin compared to contact dried blood meal ($p < 0.001$), whilst crude protein content and *in vitro* digestibility coefficients were similar (Table 1). Moreover, protein digestibility was similar between the RD (93 0.3%) and 95PHS-diet (94 0.8%), but significantly lower in the BM-diet (74 1.5%, $p<0.001$). Seen the large discrepancy observed between *in vitro* and *in vivo* protein digestibility, the pepsin-HL method proved to be a non-reliable method to asses digestible protein content of ingredients for aquafeed.

Table 1. *In vitro* (iv) and *in vivo* (rt, rainbow trout) protein digestibility (mean ± sem) of blood by-products.

Ingredient	CP (%)	iv aD_CP (%)	iv DP (%)	rt aD_CP (%)		rt DP (%)	
Actipro® 95PHS	92.9	100.0	92.9	94.0[a]	2.1	87.3[a]	2.0
Blood meal	91.3	97.3	88.9	49.5[b]	3.9	45.2[b]	3.6

CP (crude protein), aD_CP (apparent CP digestibility coefficient), DP (digestible protein) **Conclusion**. Spray dried hemoglobin showed to be a suitable ingredient to partially replace fish meal in feed for rainbow trout. High amounts of indigestible protein in contact-dried blood meal, in contrast, would deteriorate feed conversion and increase the amount of nitrogen in culture water as well as the systems' effluent.

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Effects of hydrolysed poultry byproduct meal in extruded diets on serum angiotensin converting enzyme activity and aldosterone in cats

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Introduction. Peptides from hydrolysed poultry byproduct proteins can inhibit angiotensin converting enzyme (ACE), which gives these peptides antihypertensive properties and potential to promote health [1]. ACE converts angiotensin I to angiotensin II, a potent vasoconstrictor and aldosterone (ALD) secretion stimulator, which increases blood pressure. Hydrolyzed proteins are also suggested to be easier digested and absorbed, which could improve protein digestibility [2]. However, the osmolarity of protein hydrolysate is higher than intact protein, and could also affect faecal characteristics [3]. This study aimed to evaluate the effects of hydrolysed poultry byproduct meal on ACE activity and serum ALD levels, as well as on nutrient digestibility and faecal characteristics in cats.

Animals, material and methods. Based on an in vitro ACE inhibitory activity assay [4], a conventional (CPM) and a hydrolysed poultry byproduct meal (HPM) were selected for this study. Two iso-energetic and iso-nitrogenous diets were formulated: CPM diet with 25.7% CPM and HPM diet with 24.7% HPM. All cats used were healthy, neutered and 5 to 6 years old (ethical approval number, CEUA 1738040618). In trial 1, effect of diet on serum ACE activity and ALD was evaluated using 8 cats (4 females, 4 males; 4.1±0.38 kg BW) in a cross-over design with 5 d adaptation and blood collection from the jugular vein on day 6. Serum was analysed for ACE activity [5] and ALD levels by a chemiluminescence method. In trial 2, apparent faecal digestibility [6] and consistency [7] were evaluated using 12 cats (6 females, 6 males; 4.0±0.72 kg BW) in a completely randomized design. Serum ACE and ALD were analysed using ANOVA with Diet as fixed effect and Cat as random effect (mixed model). Data from trial 2 were submitted to ANOVA and means were compared by Tukey's test. Significant differences were considered for $p < 0.05$ and tendency for $p < 0.10$.

Results and discussion. The in vitro ACE inhibitory activity of HPM (90.4%) was higher than the CPM (52.0%). In vivo, feeding cats the HPM diet tended to lower serum ACE activity when compared to the CPM diet (126.1±12.7 versus 142.1±21.3 U/L; $p = 0.09$), which is in line with the in vitro findings and with those in hypertensive rats fed chicken hydrolysate (21% decrease in plasma ACE activity) [1]. Serum ALD was not influenced by diet (Fig. 1). Diets had similar digestibility values and faecal consistency score tended to be more firm when cats were fed the CPM than HPM diet (4 versus 4.6; $p = 0.09$).

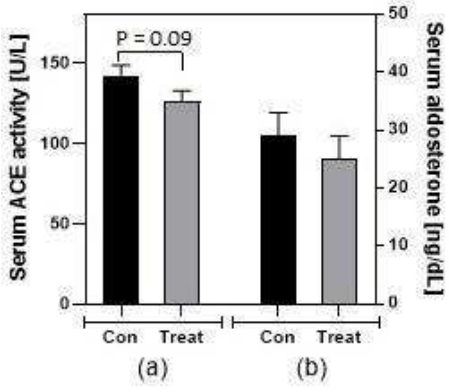

Figure 1. Average of serum (a) ACE activity (U/L) and (b) aldosterone levels (ng/dL) of cats fed with Con and Treat diets. Error bars indicate SE.

Conclusion. Including HPM in extruded diets for cats tended to reduce ACE serum activity without affecting digestibility and resulting in suitable faecal score. Further investigations are needed to explore potential health benefits in hypertensive cats.

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Nutritional characteristics of oil hemp cake

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Introduction. The industrial hemp (*Cannabis sativa* L.) is a multiuse crop cultivated to obtain different products [1]. In recent years, the interest in hemp oil was increased, and different extraction processes were developed. Several beneficial effects on human health were recognized to hemp oil due to its favourable fatty acids profile (high PUFA) [2]. In function of process used for oil extraction the remaining cake could have different nutritional characteristics [3]. The aim of this study was to evaluate the chemical and nutritional characteristics of different hemp oil cakes obtained by high pressure extraction process using the *in vitro* technique.

Animals, material and methods. Eight samples of hemp oil cake of two botanical cultivars (Futura 75 and Uso 31, 4 samples per cultivar) were studied. Two methods of high-pressure extraction, different for the nozzle size diameter (6 vs. 8 mm) were used [4] according to the procedures usually adopted by the hemp oil company which provided the samples. The chemical composition was analysed according to official methods [5,6]. To study the *in vitro* fermentation characteristics the samples were incubated with bovine buffered rumen liquor for 48 hours at 39°C under anaerobic condition [7] in order to determine the cumulative gas production, the digestibility and the fermentation kinetics. The statistical analyses (ANOVA) was performed in order to evaluate the differences between cultivars and extraction methods [8].

Results and discussion. The hemp oil cake from the larger nozzle diameter showed higher ether extract content (10.39 vs. 8.58 % DM, for 8mm and 6mm, respectively; $p<0.001$). The crude protein and NDF showed high values (25.05 and 58.81 % DM, respectively) as reported in a previous note [9]. The in vitro results indicate that after 48 hours of incubation the process was completed due to the use of all fermentable nutrients.

Comparing the cultivars significant differences were observed: Uso 31 showed higher digestibility and potential gas production values ($p<0.001$). The oil extraction methods also significantly affected the fermentation kinetics: faster for 6 mm compared to 8 mm cake. These differences could be due to the lipid amount which has interfered in the fermentation processes.

Conclusion. The data obtained in this preliminary study evidence as, despite the high amount of NDF and crude protein, the use of this co-product in ruminant nutrition needs to be carefully evaluated due to the high fat values, which can limit ruminal microbial activities.

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Hemp oil cake: chemical composition according to traditional and near infrared spectroscopy (NIRs) methods of analysis

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Introduction. Hemp (*Cannabis sativa* L.) was traditionally grown as a source of fiber, but recently the interest in this plant was renewed for food production [1]. In particular, the seed oil received several attentions for its nutritional properties and health benefits [2]. Furthermore, the main co-product of oil extraction, the cake residual, could be used as animal feed due to the concentration of several nutrients [3]. The aim of this study was to evaluate the chemical composition of hemp oil cake using two analytical approaches: the traditional methods and the near infrared spectrophotometry (NIRs).

Animals, material and methods. The samples were collected in a hemp processing plant located in province of Napoli (Italy), where the hemp seeds are subjected to high pressure oil extraction. In particular, eight samples of hemp oil cake obtained from two botanical cultivars (Futura 75 and Uso 31, 4 samples for each cultivar) were analyzed using Weende and Van Soest [4,5] methods and NIRs (NIRFlex N-500, Buchi Instruments Inc). All the data were analyzed statically [6]: to evaluate the differences between cultivars (Futura 75 *vs*. Uso 31) and methods of analyses (traditional *vs*. NIRs) two sperate ANOVA test were carried out and to verify the efficacy of NIRs method the correlation with the traditional method was performed.

Results and discussion. All analyzed samples showed high concentration of crude protein (>20% DM) and NDF (>40% DM) according to other studies [8]. Despite the oil extraction, the values of ether extract were higher than 7% DM. In general, the cultivar had no effect on the tested parameters, even if some differences were observed in function of the used method. In fact, the values of crude protein and NDF registered for Futura 75 were significantly higher ($p<0.01$) when the traditional method of analysis was used. Comparing the methods, the crude protein (25.05 vs. 22.22 % DM, $p<0.001$) and NDF values (58.81 vs. 46.22 % DM, $p<0.001$) resulted higher when analyzed with traditional method, whereas lipid content was higher with NIRs technique (10.95 vs. 9.48 % DM, $p<0.01$). Evaluating the correlation between the analytical methods, some significances were observed for crude protein (r 0.9398; $p<0.001$) and ether extract (r 0.796; $p<0.001$).

Conclusion. The data obtained by both analytical methods provide interesting information about the chemical and nutritional properties of hemp oil cakes. In particular, their high protein and NDF contents suggest their potential use in herbivores' nutrition.

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A120

HO-proline is not a good marker of protein digestibility

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Introduction. Hydroxyproline (a marker of collagen) is commonly used to assess dietary protein quality in petfood. Protein quality depends on its digestibility and essential amino acid profile. Although the amino acid profile of collagen is poor, the blend of other animal and plant proteins in the formula allows to balance it easily. The aim of this study was to assess the digestibility of collagen rich pork greaves in dogs.

Animals, material and methods: Digestibility trials were performed on 10 large breed dogs according to AAFCO protocol (10 days). Two extruded diets with the same nutritional profile (Crude protein 27%, Crude fat 16% and TDF 6%) and meeting AAFCO maintenance requirements were fed. Poultry meal and pork greaves (89 and 95% in vitro Boisen digestibility) were used for the control and test diets at 12 and 10% inclusion respectively. HO-proline was 0.55% and 1.16% as fed in the control and test diets. Feces were collected over 5 days and analyzed for dry matter, protein and energy. A 20g samples of fresh feces, was used to measure H_2S and mercaptan by electrochemical diffusion (Dräger sensor, Dräger, Lübeck, Germany). An expert panel was used to score fecal odor on fresh feces. Fecal scores were monitored during the feces collection[1]. The protocol was accredited by the French Ministry of Research (APAFIS#18586-201911116181974v3). Results were compared by unpaired student t-test; Significant differences were considered for $p < 0.05$. Results are expressed by mean±SD.

Results and discussion. Fecal scores were improved on the pork greave diet. Apparent protein digestibility was 88.2±1.6% and 91.4±1.5% for the control and test diets. Figure 1 shows that using pork greave protein at 10% inclusion reduces significantly sulfur compounds in fresh feces. This reduction was linked to a reduction in the fecal odor score by the expert panel. Those observations support a reduction in colonic protein fermentation with collagen rich pork greaves

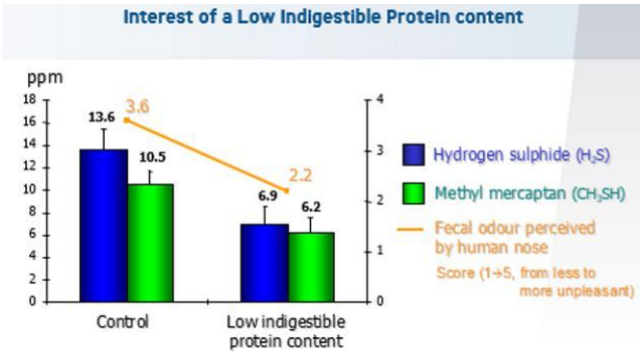

Figure1: Comparison of H2S and Methyl mercaptan released by 20 g of fresh feces

Conclusion. Contrarily to a common belief, collagen from pork greaves is highly digestible and decreases colonic fermentation compared to poultry meal. Hydroxyproline is not a good marker of protein digestibility in diets made with this raw material. The origin of the material is key to assess the quality of a petfood.

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Do Brazilian vegetarian or vegan pet owners transfer their eating habits to their pets?

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Introduction. Human eating habits are in growing transition to vegetarian or vegan diets and many of them have dogs and cats. Brazilian pet food companies take on these consumers tend to extend their feeding habits to their pets and, seizing this market opportunity, develop extruded vegan and vegetarian foods for pets. However, little is known about this market niche in Brazil. Thus, the objectives of this study were to verify whether the owner's vegetarian or vegan habits influence their pets' eating habits and to estimate the population of dogs and cats that receive this type of diet in Brazil.

Animals, material and methods. An online questionnaire was released to vegetarian/vegan Brazilian pet owners. This questionnaire was developed with Google Forms and disseminated online through social media. The set of questions consisted of basic information about owners, animal species, types of food fed to pets, and other information related to pets (outside access, *ad libtum* feeding, difficulties on feeding them with vegetarian/vegan food, their opinion about health and well-being related to this type of diet, among others). Categorical responses were analyzed using descriptive statistics. Quantitative and graphical data were developed in an Excel 2013® spreadsheet, reported as frequency (n) and percentage (%).

Results and discussion. Altogether**,** 229 pet owners answered the questionnaire. Of these, 229 were vegetarian/vegan, and 12.23% offered their animals a vegetable-based diet, being 13.79% cat owners, 48.27% dog owners and 34.48% both dog and cat owners. 34.48% owned both dogs and cats with these eating habits. These participants believed that their pet may have vegetarian/vegan eating habits (2.62%) by itself at home, and only about 3.49% of them thought that this type of food is healthier. However, studies have shown that there are nutritional deficiencies in homemade [1] and industrialized [2] vegetarian/vegan foods, mainly of calcium, calcium:phosphorus ratio, methionine, taurine, arachidonic acid, and potassium [2]. It is known that some of these deficiencies can impair the animal's health [3]. From the animals that received vegetarian/vegan food, 55.72% are not taken frequently to the veterinarian for exams. In addition, 46.15% of owners observed that their pets have difficulties on eating this type of food.

Conclusion. The majority of dogs and cats of vegetarian or vegan owners are not fed, which demonstrates that the eating habits of most vegetarian and vegan owners do not influence their pets' feeding habits. Nevertheless, this type of diet is associated to health risks of pets.

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Determining praecaecal, postileal and total tract digestibility of rye based diets when substituting soybean meal with rapeseed meal in Göttingen minipigs

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Introduction: Currently compound feeds for pig nutrition contain high proportions of wheat and soybean meal (SBM) while the levels of rye and rapeseed meal (RSM) are low but certain advantages of rye and rapeseed are worth to be considered [1,2]. Previous investigations (with different shares of either wheat or rye in compound feeds) concluded that substituting wheat with rye in high shares does not result in reduced performance for pigs when the diets are supplemented adequately [3]. Therefore, it was of interest to determine effects on apparent precaecal (AID), postileal (APD) and total tract (ATTD) digestibility of two rye based diets (60 % in general) with high shares of either RSM or SBM in adult minipigs.

Animals, materials and methods: Four adult Göttingen minipigs (30.7±1.63kg; 4.3±3.3yrs) were fed two different diets containing 60% rye, *Secale cereale* as well as either 18.1% SBM (F1) or 28% RSM (F2), barley and a mineral/amino acid supplement to reach comparable nutrient values. The individually housed pigs were fitted with an ileo-caecal fistula. The diets were ground finely (0.5mm sieve insert) and fed to the pigs in two servings of each 200g (+ 1l of water and 0.625g of Cr_2O_3) a day. After adaptation (7d), faeces were collected individually (5d), then digesta collection (3d) started, during which the pigs were fed at 7:00h and the fistulae were opened. For the following 12h the whole ileal digesta was collected. The dry matter content of digesta and faeces were measured, then crude nutrients were analysed in the lyophilised material. AID was calculated with the marker method and ATTD with the collection method. Statistical analyses were performed using the SAS® software (ANOVA).

Results and discussion: Significant differences ($p<0.05$) were found for AID of organic matter (OM) and N-free extractive (NfE) and for ATTD of OM, crude protein, crude fiber and NfE, i.e. higher values for the SBM- than for the RSM-based diet.

Table 1: AID and AATD of nutrients (in %) of the two diets based on rye, supplemented by SBM or RSM

Site	Diet	OM	XP[1]	EE[2]	XF[3]	NfE
AID	F1 (SBM)	74.3^{a}±1.43	77.2±7.27	78.0±5.60	15.6±5.03	77.5^{a}±4.56
	F2 (RSM)	66.0^{b}±1.97	73.3±1.95	85.5±2.56	11.5±6.18	67.4^{b}±3.26
ATTD	F1 (SBM)	90.2^{A}±1.19	91.4^{A}±1.38	77.5±3.56	57.0^{A}±3.78	92.9^{A}±0.946
	F2 (RSM)	84.8^{B}±2.55	84.6^{B}±2.92	79.7±4.18	46.4^{B}±7.67	89.1^{B}±1.85

^{a,b}: significant differences ($p<0.05$) for AID in a column; ^{A,B} for ATTD in a column; [1]crude protein, [2]ether extract, [3]crude fibre

Calculating the postileal digestibility from the differences between AID and ATTD, F2 reached 1.09-, 1.17- and 1.44-fold higher values for OM and NfE respectively and F1 1.30- and 1.16-fold higher values for XP and XF.

Conclusion: As AID and ATTD of F2 were significantly lower for most nutrients, it can be concluded, that substituting SBM with RSM in high shares of a rye-based diet will lead to a higher FCR. However, APD was higher for most of the values in F2, so it may contribute to forced formation of butyrate and prolonged feeling of satiety [1] due to increased fermentation, effects that might be used in feeding pregnant sows.

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Survey: puppy feeding practices and perceptions in Switzerland

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Introduction. Feeding dogs homemade diets has gained popularity. When done inappropriately, this renders an increased risk of nutritional imbalances and infections [1]–[3]. Surveys have highlighted the resulting incidence of malnutrition in pets [1], [3] and puppies in specific [4]. We hypothesised that opinions regarding puppy feeding, decision criteria, general knowledge and sources of information differ between six age categories of visitors at a dog exhibition.

Animals, materials and methods. The survey was conducted at an annual dog exhibition in Switzerland. 15 multiple choice questions were asked: 5 about the applicant (AP), 2 about information sources, 8 about puppy feeding. 117 AP completed the survey of which aged 8 (7%) <25 (G1), 20 (17%) 25-35 (G2), 14 (12%) 35-45 (G3), 37 (32%) 45-55 (G4), 29 (25%) 55-65 (G5), 9 (8%) >65 (G6) years (set age groups). About 93(±4)% (average ± standard deviation) of AP owned a dog (except G1: 50%). 63%, 50%, 39% and <22% of G1, G5, G2 and G3&4&6 respectively, were professionally involved with dogs (e.g. veterinarian, breeder, dog walker). 43(±6)% of AP did some leisure activity with their dog.

Results and discussion. Where not specified, descriptive statistics were calculated over all AP instead of per age group. The veterinarian comprised the primary source of information regarding puppy development (65±10%), except in G6 where 67% preferred their breeder. In G1&3&4 the veterinarian was also the main source on puppy feeding (63%; 50%; 35%). In G2 AP relied more on their own experience (33%) whereas in G5&6 (33%; 60%) the breeder was preferred. Only 1% of AP stated the internet as primary source. Most AP of all age groups preferred to feed dry food (puppy: 83(±10)%, adult: 82(±7)%). Apart from G3 (14% and 7%) and G6 (2x13%), AP considered wet food secondly (puppy: 39±8%, adult: 29±7%). In all age groups except G5 (29%), less than 14% preferred to cook. No one in G1&3&6 preferred raw food for their puppy, compared to 20%, 24% and 11% in G2&4&5. For adult dogs this increased to 25%, 7%, 27% and 18% in G2&3&4&5. On the label of puppy food, 60(±12)% of AP stated “for growing dogs” as important, followed by “grain free” 26(±13)%, “organic” 26(±15)%, “for all ages” 23(±13)%, “regional” 11(±6)% and “for adult dogs” 9(±7)%. Most AP of all age groups correctly preferred a puppy diet to contain a limited variety of feedstuffs (83±8%), meat as the main protein source (77±14%) and to ensure a slow growth (98±3%). Only 24(±13)% stated supplements for puppies as necessary depending on the diet. AP considered protein (61±15%) followed by calcium (43±25%), vitamin A (31±18%), vitamin D (21±12%) and B-vitamins (13±12%) harmful in excess to puppies.

Conclusion. The results of this survey agree to the importance stated elsewhere of educating veterinarians and breeders as primary sources of information [1], [4].
In addition, it implies the further use of age groups in similar surveys due to apparent opinion differences between generations.

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Session 5 Pre- and probiotics & General

Chair: Anne Möβeler

Impact of varying dietary concentrations of dried food residues on feed intake, apparent nutrient digestibility and fecal bacterial metabolites of adult dogs

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Introduction. The project "Food for Feed" (LIFE15 ENV/GR/000257) evaluates the use of dried food residues (DFR) from hotel catering in animal nutrition. The approach could contribute to a significant reduction of waste production and environmental burden by food waste, though legal restrictions are still a hurdle. In the present study, the use of DFR as a component for dog food was evaluated.

Animals, materials and methods. Ten healthy adult dogs were fed a diet with varying amounts of DFR (0 %, 5 %, 10 % and 15 %). The food residues were collected in hotels in Crete, ground and solar-dried. The same batch of DFR (Table 1) was used as an ingredient for the experimental diets, which were based on poultry meal and rice flour. For the determination of apparent nutrient digestibility, titanium oxide was mixed to the diet at 0.2 %. The diets were fed for three weeks each. Daily amount of feed was calculated and weekly adjusted to maintain body weight (BW). Feed intake was recorded daily. At the end of each feeding period, fresh fecal samples were collected. Polynomial contrasts were calculated for group comparisons (GLM repeated measures; SPSS 22), with $P < 0.05$ as level of significance.

Table 1: Dry matter (DM), crude nutrient and metabolizable energy (ME) concentration of the DFR and the experimental diets

		DFR	Complete diets with DFR at			
			0 %	5 %	10 %	15 %
DM	g/kg	912	929	928	926	925
Crude protein	g/kg DM	259	249	249	251	248
Crude fat	g/kg DM	247	99.4	99.4	94.9	98.7
Crude fibre	g/kg DM	34.6	9.47	9.16	8.96	9.19
Crude ash	g/kg DM	59.7	44.0	47.8	47.1	46.4
Calculated ME[1]	MJ/kg	-	17.2	17.1	17.0	17.1

2) NRC (2006)

Results and discussion. No adverse reactions related to the DFR were observed in the dogs. The daily amount of feed had to be increased at the highest dietary inclusion level of DFR in order to maintain BW of the dogs (Table 2). This might be probably attributed to the reduced apparent digestibility of crude protein and crude fat with increasing amounts of DFR in the diets. Fecal ammonia and L-lactate concentrations were not affected by the diets. Interestingly, the lowest amount of feed to maintain BW was required when the diet 5 % DFR was fed. Also, in this group, highest fecal D-lactate concentrations were measured.

Table 2: Body weight (BW) (kg), feed intake (g dry matter/kg BW/day), apparent digestibility (AD) (%), fecal ammonium (µmol/g) and lactate concentrations (µmol/g) in dogs fed a diet with varying amounts of dried food residues (DFR). Means and SEM

	Complete diets with DFR at				SEM	Polynomial contrasts (*P* value)	
	0 %	5 %	10 %	15 %		Linear	Quadratic
BW	12.4	12.7	12.4	12.5	0.18	0.542	0.304
Feed intake	18.4	16.8	18.1	19.4	0.24	**0.008**	**0.001**
AD CP	81.5	83.4	78.1	74.0	1.26	**0.008**	0.161
AD EE	93.9	93.8	89.7	92.8	0.54	**0.006**	0.339
Fecal ammonium	31.5	36.5	36.2	34.6	1.31	0.501	0.242
Fecal L-lactate	0.01	0.29	0.01	0.01	0.05	0.126	0.131
Fecal D-lactate	0.02	0.13	0.06	0.04	0.02	0.911	**0.045**

CP: crude protein; EE: crude fat

Conclusion. DFR might be a potential component of dog food, particularly at lower dietary inclusion levels (5 % or less). **Reference:** 1 National Research Council (NRC). Nutrient Requirements of Dogs and Cats. Washington,DC: The National Academies Press; 2006.

Safety, gastrointestinal tolerance, and utility of a novel animal milk oligosaccharide biosimilar in healthy adult cats

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Introduction: In mammals, milk is an excellent source of nutrition to ensure adaptation to the extra-uterine life. Among the bioactive components, milk oligosaccharides (MOs) act as prebiotics, antiadhesives, and antimicrobials having an effect in altering epithelial and immune cell response. MOs serve selectively as a source of energy for desired bacteria that are important in the gut for modulating mucosal barrier function, promoting immunological responses and avoiding the adhesion of certain pathogens. We have developed an animal milk oligosaccharide biosimilar (NXC139) that was shown to increase the production of short-chain fatty acids (SCFA) and the growth of beneficial microbiota in an *in vitro* study using cat stool inoculum. To evaluate the safety, gastrointestinal tolerance, and utility of this novel animal milk oligosaccharide biosimilar, 24 healthy adult cats were fed with NXC139. **Methods:** After an acclimation phase receiving a control diet for 14 days, 8 cats were randomly assigned to different groups and fed for 28 days. We report on control group (0% NXC139) and 2 treatment groups (1% or 1.5%, NXC139). Fresh fecal samples were collected after 14 and 28 days for measurement of fecal metabolites [phenol, indole, short-chain fatty acids (acetate, propionate, butyrate) and branched-chain fatty acids (isobutyrate, isobutyrate, isovalerate, valerate) and ammonia]. DNA was extracted from the fecal samples and was processed using the Pet-16Seq workflow for 16S rDNA bacterial detection. After demultiplexing, sequence quality control and paired-end reads merging, amplicon sequence variants (ASVs) were generated using the Divisive Amplicon Denoising Algorithm (DADA2). Paleontological Statistics (PAST; v3.12) software was used for alpha-diversity analyses including richness (ASVs), Shannon diversity index and evenness. The significant differences were calculated using Mann–Whitney U test in PAST; v3.12. $p < 0.05$ were considered statistically significant. **Results:** There was a non-significant but numerical decrease of the total amount of phenol and a decrease with statistical differences of the total amount of isovalerate in fecal samples of cats receiving 1% NXC139 in comparison with the control group after 28 days. Also, there was a non-significant but numerical increase of the total amount of butyrate in fecal samples of cats receiving 1.5% NXC139 in comparison with control group after 28 days. There was an increase of the normalized read count of important genera such as *Bifidobacterium*, *Ruminococcus* or *Blautia* and a decrease of genera which include potential pathogenic bacteria such as *Campylobacter*. **Conclusion:** The study showed that NXC139 appears to beneficially modify the gut microbiota of cats, enhancing energy and metabolic homeostasis and supporting gut health increasing the amount of SCFA and decreasing phenol and isovalerate. Moreover, it seems to promote a pathogen inhibitory effect and a selective commensal stimulation effect in the gut microbiota of an adult cat. Furthermore, current biomarkers highlights safety and good tolerability of NXC139.

Can Diet Shift the Changes of Gut Microbiota of Dogs Closer to Wolves?

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Introduction. The genomic signature of dog domestication reveals adaptation to a starch-rich diet compared to their ancestor wolves. Diet is a key element to shape gut microbiota. Few studies have compared gut microbiota between dogs and wolves [1]. The present study aimed to investigate the dynamic changes of gut microbiota of dogs from processed diet (kibble) to raw meat diet compared with wolves.

Animals, material and methods. Six healthy wolves (W) from Yancheng Wild Zoo and six healthy American Staffordshire Terriers from Anbei Pet Food Company were included (EC number: NXY2018/01). Wolves were housed and fed individually during the day of sampling. Wolves were fed their typical raw meat diet. Dogs were housed individually and fed the same dry diet for at least three month before sampling at day 0 (DC), and then switched to raw meat diet (the same diet as wolves) for 28 days. Samples from the dogs were collected at day 1 (DR1), week 1 (DR2), 2 (DR3), 3(DR4), and 4 (DR5). Six fresh fecal samples from the wolves and 36 fresh fecal samples from the dogs were collected. Fecal microbiota were analyzed using 16S rRNA gene 454-pyrosequencing.

Results and discussion. Principle component analysis (PCoA) showed that changing the diet from processed to raw meat in dogs shifted the gut microbiota closer to that of wolves on PC1, however, low percentage of explanation should be noted (Fig. 1). At phylum level, Acidobacteria were significantly higher in wolves while almost non-existent in dogs regardless of the diet. Acidobacteria is a major taxonomic group in soil, their function including nitrite and nitrate reduction [2]. Moreover, raw meat consumption increased abundance of Fusobacteria and Bacteroidetes at DR1, DR2, DR3 and DR4 (P<0.05) compared to DC, where as no difference were observed between DC and DR5. This might suggests that the abundance of these phyla tended to stabilize and return to the starting point after 4 weeks.

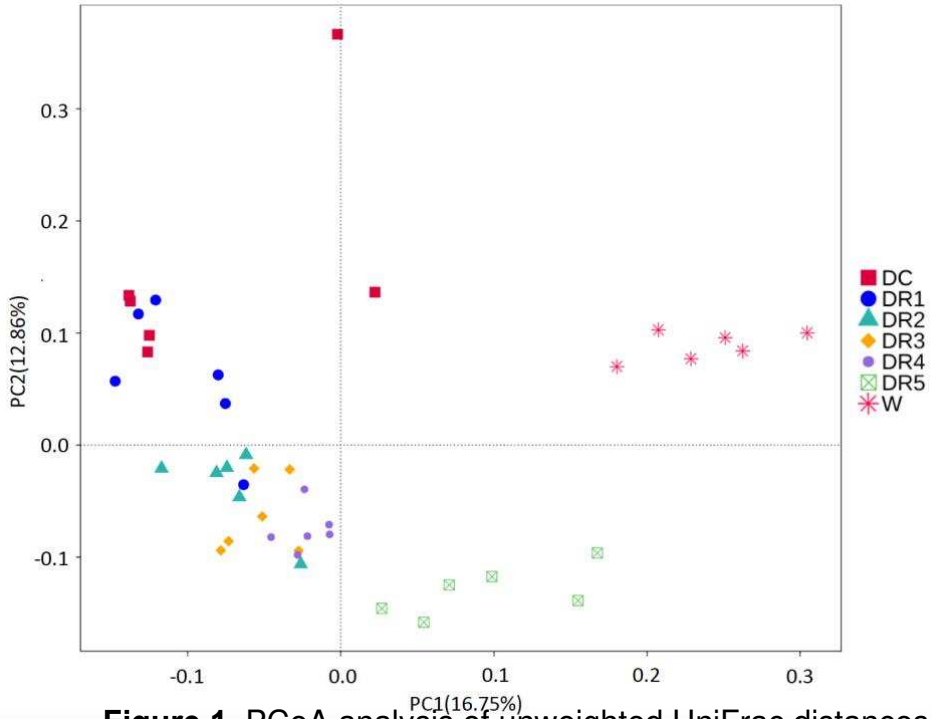

Figure 1. PCoA analysis of unweighted UniFrac distances of 16 S rRNA genes.

At genus level, Faecalibacterium, Butyricicoccus, Turicibacter, Catenbacterium, Allisonella and Megamonas were lower in wolves and dogs consuming raw meat diet compared to dogs on the processed diet. Among them, Faecalibacterium, Butyricicoccus, Turicibacter have been positively associated with gut health [3].

Conclusion. The change of diet from processed dry food to raw meat has a tendency to shift the gut microbiota of dogs closer to that of wolves.

References: [1] Alessandri et al. (2019) Environ Microbiol. 21:1331-43; [2] Kielak et al. (2016) Front Microbiol. 7:744; [3] Rossi et al (2014). PLoS One. 9:e94699.

Mixed fungal-bacterial inoculum on alfalfa (Medicago sativa L.) crop: effects on forage proximate and fatty acid compositions

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Introduction. Microbial inoculants are biofertilizer formulations composed of beneficial microorganisms supporting plant growth and health that play a key role in sustainable crop production [1]. The use of biofertilizers on alfalfa (Medicago sativa L.) crops has been shown to improve plant physiology, nutrient uptake, growth and yield under arid climatic conditions [2]. Scant information is available on the effects of microbial inocula on alfalfa nutritive value. The "MicroBOOST" project investigated the impact of a fungal-bacterial inoculum on the proximate and fatty acid (FA) compositions of alfalfa. **Material and methods.** Two adjacent experimental alfalfa (var. Padus) fields (sandy-loam and silt-loam soil conditions) were used (lat.: 44°75' N, long.: 7°60' E; alt.: 260 m a.s.l.). Both fields never received any microbial inoculum before the beginning of the trial. One field (MI) was inoculated (Micosat F, encompassing saprophytic and symbiont fungi, and rhizosphere bacteria; CCS-Aosta S.r.l.) while the other field was not inoculated and used as control (CTRL). Three forage samples from each field were collected at three time points across the vegetative season (T1: 6/6/2019; T2: 6/8/2019; T3: 9/10/2019) and analyzed for their proximate and FA compositions [3]. Data were subjected to Two-Way ANOVA, considering the fixed effects of treatment (TR), time (T) and their interaction (TR×T).

Results and discussion. The performance of biofertilizers is expected to depend on the strain(s) used, soil and climatic conditions. In our conditions, inoculation did not significantly affect the proximate constituents nor the main FA in the aerial part of the plant (Table 1). As expected, sampling time (related to differences in plant phenology and harvesting time) had a major impact on all the considered dependent variables. The interaction between treatment and time was never significant.

Table 1. Proximate composition (g/100g DM) and main FA (g/kg DM) of alfalfa inoculated (MI) or not (CTRL) with a commercial fungal-bacterial inoculum.

	CTRL			MI			Significance		
Item	T1	T2	T3	T1	T2	T3	TR	T	TR×T
DM	21.97	21.80	18.95	23.22	23.24	18.76	ns	**	ns
Ash	7.70	8.53	8.83	8.07	8.93	9.16	ns	*	ns
CP	20.27	21.87	23.60	21.28	21.83	23.48	ns	**	ns
EE	3.27	4.00	4.45	3.38	4.13	4.50	ns	***	ns
NDF	38.96	33.09	22.78	38.78	30.09	22.53	ns	***	ns
C16:0	4.11	5.21	5.43	4.13	5.30	5.60	ns	***	ns
C18:0	0.64	0.89	0.80	0.69	0.95	0.82	ns	***	ns
C18:1 c9	0.36	0.52	0.26	0.34	0.55	0.31	ns	***	ns
C18:2 n6	4.81	5.02	6.52	4.67	5.00	6.45	ns	***	ns
C18:3 n3	14.84	18.67	23.52	15.52	18.75	24.81	ns	***	ns

*p < 0.05; **p < 0.01; ***p < 0.001.

Conclusion. In our hands, microbial inoculum did not significantly affect proximate and main FA constituents of alfalfa grown in sandy-loam and silt-loam soils under humid temperate climatic conditions.

References: [1] Itelima et al. (2018) Microbiol. Biotechnol. Rep. 2: 1; [2] Daur et al. (2018) Front. Microbiol. 9: 477; [3] Ravetto Enri et al. (2017) J. Sci. Food Agric. 97: 1252-9.

Prebiotic effects of fermented liquid feed (FLF) supplemented with 40% of non-fermented coarse, roller mill ground cereals

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Introduction: FLF is characterized by pH values below 4.5, high counts of lactic acid bacteria (> 9 log10 cfu/g diet) and high amounts of lactic acid [1]. We hypothesised that introducing high amounts of lactic acid bacteria into the pig's gastrointestinal tract via FLF could have an impact on the intestinal bacterial ecosystem. Whereby FLF containing non-fermented coarse cereals could act as prebiotic and stimulate the growth of one or a limited number of potentially health-promoting indigenous microorganisms. The aim of the present study was to investigate intestinal and faecal microbiota of young fattening pigs offered FLF supplemented with non-fermented coarse, roller mill ground cereals.

Animals, materials and methods: A total of 20 crossbred piglets were obtained at the age of 61 ± 2 days (mean body weight of 20.8 ± 2.06 kg) and allocated to four dietary treatments (two groups per experiment with five animals each). The botanical composition of all diets was identically consisting of rye, REM (rapeseed extracted meal), barley, wheat and a mineral supplement. In Experiment 1 the components of the diets (except REM) were ground by hammer mill and mixed with water immediately before feeding, i.e. non-fermented (= common liquid feed, NF) or fully fermented (FF). Only 60% of the experimental diet in Experiment 2 was fermented (REM and a part of the rye), whereas 40% of the diet consisting of rye (50%) as well as barley (25%) and wheat (25%) were ground by roller mill, but not fermented (partially fermented, PF^+). As control a non-fermented, common liquid feed similar to Experiment 1 was offered. The diets were offered *ad libitum* fresh twice a day for 28 days.

Table 1. Lactic acid bacteria counts (log10 cfu/g diet), lactic acid amount and pH of the diets as well as Geometric Mean Diameter (GMD) [2] measured by wet sieve analysis

	Experiment 1		Experiment 2	
Diet	**NF**	**FF**	**NF**	**PF^+**
Lactic acid bacteria	4.91	9.31	4.96	9.05
L-lactate (g/kg DM)	0.103	26.2	0.104	16.7
D-lactate (g/kg DM)	0.052	27.5	0	15.5
pH	5.95	3.75	5.95	3.95
GMD	476	203	439	470

Microbiota studies were performed on samples of the small and large intestine and feaces. 16S rRNA gene amplification was performed within the hypervariable region V4 and sequenced with Illumina MiSeq platform. Statistical analyses were done in R (3.5.2).

Results and discussion: The factor diet contributed significantly to the differences in microbiota composition of intestinal contents explaining 21.4% of the sample's variability. The digesta of the small intestine of pigs fed FLF were dominated by *Lactobacillaceae* (rel. abundance up to 95%). In colonic contents, the abundance of the family *Lactobacillaceae* was significantly higher only in pigs fed the PF^+ compared to NF in Experiment 2 (padj = 0.0476). Two OTUs belonging to the genus *Lactobacillus* (LB) and *Bifidobacterium* (BB) were significantly enriched in small intestine (LB: padj < 0.0001; BB: padj < 0.001) and colon digesta (LB and BB: padj < 0.0001) as well as in faeces (LB and BB: padj < 0.0001) only in pigs offered PF^+ compared to pigs offered NF in Experiment 2.

Conclusion: In addition to the expected probiotic effect, feeding FLF supplemented with 40% of non-fermented coarse, roller mill ground cereals had impacts on bacterial composition of the entire intestinal tract comparable to a prebiotic.

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GO TO ABSTRACT LIST

Dietary fiber in sows and its impact on piglets' susceptibility to *Clostridioides difficile* gut infection

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Introduction. Disruption of the natural microbial colonization process or perturbances of the intestinal ecosystem may enhance the susceptibility to *Clostridioides difficile* infections (CDI) in neonatal piglets [1]. The sow-piglet association during early life is a critical factor for the development of the gut microbiota and the immune system and could have an impact on the susceptibility of neonatal piglets to CDI. Dietary fiber is an attractive feed ingredient able to modulate the gut microbiota with a subsequent influence on host physiology and health [2]. The authors hypothesized that a maternal diet rich in high- or low-fermentable fiber during gestation and lactation changes the gut microbiota and physiology in sows, which further influences the susceptibility to CDI in neonatal piglets. **Animals, material and methods.** Twenty sows were fed experimental gestation and lactation diets enriched with either sugar beet pulp (T1; inclusion rate: 15 % sugar beet pulp, 3 % lignocellulose; n=10 sows) or lignocellulose (T2; inclusion rate: 15 % lignocellulose, sugar beet pulp; n=10 sows). The diets were based on barley, wheat and soybean meal. Gestating sows were kept in groups and housed individually one week ante-partum until weaning at day 28. The sows were fed restrictively during gestation, while ad libitum during lactation. Colostrum was collected from the sows within 12 hours post-partum and assessed for crude protein and crude fat (Weende standard procedures [3]), lactose (enzymatic assay), immunoglobulins (ELISA) and biogenic amines (ion-exchange chromatography). One average-body-weight neonatal piglet from each sow was euthanized between 4 and 6 days of life and examined for mesocolonic edema (score: 1=normal, 2=mild, 3=moderate, 4=severe). Further, feces and colon contents were assessed and *C. difficile* spore count (selective agar plates) and toxin B (TcdB) levels (ELISA) were measured. The "Bristol stool form scale" was used to assess the fecal score [4]. Data were analyzed by Mann-Whintey U test and Spearman's rho correlations (SPSS version 24.0.0.0). The animal trial was approved by the Regional Office for Health and Social Affairs (LaGeSo Reg. G0112/19). **Results and discussion.** There were no differences between treatments regarding litter size at birth, average piglet weight at birth or at weaning, and average piglet weight gain. In colostrum, crude protein content (dry matter) was significantly higher in T1, as compared to T2 (198.9 g/kg ± 21.9 vs. 176.6 g/kg ± 14.9, $p=0.021$), while the concentrations of crude fat, lactose, immunoglobulins and biogenic amines did not differ between the dietary groups. Levels of crude protein and lactose showed a negative association (rho=-0.816, $p<0.0001$), which is consistent with published reports [5]. Piglet fecal and colon pathological scores, concentrations of *C. difficile* and TcdB did not differ between the study groups. The piglets did not show any clinical signs of CDI. There were several strong positive correlations between fecal and colon *C. difficile* and TcdB concentrations and colon clinical scores, demonstrating that the gut of neonatal piglets is colonized by virulent *C. difficile* which may be involved in mesocolonic edema development. **Conclusion.** Diets enriched with high- or low-fermentable fiber sources have a moderate impact on specific colostrum components in sows. The impact of fiber on the sow-piglet association and the susceptibility of the neonatal piglet to *C. difficile* colonization and infection needs to be elucidated and further analyses are warranted.

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Effects of scFOS supplementation on putrefactive compounds in cats: a systematic review.

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Introduction. Cats as obligate carnivores are fed with diets high in proteins; they are metabolically adapted to preferential use of protein as an energy source. However, some dietary proteins reaching the colon are fermented by the microbiota, leading to the accumulation of fermentation products responsible for malodorous faeces and gut disorders [1]. A dietary supplementation with short-chain fructo-oligosaccharides (scFOS) defined as prebiotic fibres may be of interest to alleviate negative impacts of protein fermentation. The objective of this systematic review was to evaluate the effects of a scFOS supplementation on the production of putrefactive compounds in cats.

Materials and methods. A comprehensive literature search was performed using the NCBI Pubmed database on putrefactive compounds in cats supplemented with scFOS. Studies were considered eligible if the following criteria were met: (1) study conducted in cats; (2) scFOS tested as single supplementary ingredient; and (3) an outcome on faecal putrefactive compounds: ammonia, cadaverine, putrescine, tyramine, total amines, indoles, phenols, or branched-chain fatty acids.

Results and discussion. From 16 studies identified, 4 studies met all inclusion criteria and have been selected [2-5]. Three studies among the 4 relevant studies have shown a reduction of putrefactive compounds concentration in faeces of cats supplemented with scFOS while the type of putrefactive compounds affected differed between studies. The differential effect of scFOS observed between studies on putrefactive compounds could be explained by a high variability intra- and inter-studies, a low number of cats, the type of analysis, diet characteristics, the dose of scFOS and the duration of the study. In addition, two studies showed a significant reduction of the intensity of faecal odours with scFOS intake.

Conclusion. This systematic review demonstrated that a supplementation with scFOS allows to reduce faecal concentrations of putrefactive compounds in cats. Reducing the production of these compounds is beneficial because of their malodourous properties and their potential negative effect on gut health. Such reduction may be achieved by balancing the gut microbiota with scFOS, as highlighted in selected relevant studies by correlation between gut microbiota composition and putrefactive compound concentrations.

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Effects of a novel animal milk oligosaccharide biosimilar on fecal metabolites and microbiome of healthy adult dogs

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Introduction: Mammal milk is an excellent source of nutrition essential for animal growth. Animal milk oligosaccharides (AMOs) are one of the most abundant bioactive components [1-3]. These unconjugated complex glycans act as prebiotics, antiadhesives, and antimicrobials and play critical roles in immune cell responses [4]. Moreover, AMOs selectively serve as a source of energy for desired bacteria that are important for mucosal barrier function, immune response and pathogen adhesion [1-4]. We have developed an AMO biosimilar (NXC139) that was shown to increase the production of short-chain fatty acids (SCFA) and the growth of beneficial microbiota *in vitro* using dog stool inoculum. The objective of this study was to evaluate the safety, gastrointestinal tolerance, and utility of a novel animal milk oligosaccharide biosimilar (NXC139) in a completely randomized design with 24 healthy adult beagle dogs. **Methods:** After an acclimation phase receiving a control diet for 14 days, the 24 dogs were randomly assigned to three different groups and fed for 28 days. We report on control group (0% NXC139, n = 8) and 2 treatment groups (0.5%, n = 8 or 1% NXC139, n = 8). Fresh fecal samples were collected after 14 and 28 days for measurement fecal score, dry matter (DM) and fecal pH and metabolites [SCFA; branched-chain fatty acids (BCFA); phenols and indoles; ammonia]. DNA was extracted from the fecal samples and was processed using the Pet-16Seq workflow for 16S rDNA bacterial detection. After demultiplexing, sequence quality control and paired-end reads merging, amplicon sequence variants (ASVs) were generated using the Divisive Amplicon Denoising Algorithm (DADA2). Paleontological Statistics (PAST; v3.12) software was used for alpha-diversity analyses including richness (ASVs), Shannon diversity index and evenness. The significant differences were calculated using Mann– Whitney U test in PAST; v3.12. $p < 0.05$ were considered statistically significant. **Results:** There was a non-significant but numerical decrease of the total amount of phenol and an increase of the SCFA in fecal samples of dogs receiving 0.5 and 1% NXC139 in comparison with control group. There was non-significant but numerical increase of the richness, evenness and Shannon diversity index after 28 days in dogs receiving 0.5 and 1% NXC139 in comparison with the control group. Moreover, there was a non-significant increase of the normalized read count of genera such as *Lactobacillus*, *Bacteroides* or *Megamonas* and a decrease of genera which include potential pathogenic bacteria such as *Actinomyces* or *Clostridium* XI. **Conclusion:** NXC139 modifies the gut microbiota of dogs moderately, enhancing energy and metabolic homeostasis and increasing the amount of SCFA and decreasing phenol. Moreover, it slightly seems to promote a pathogen inhibitory effect and a selective commensal stimulation effect in the gut microbiota of adult dogs. Furthermore, current biomarkers highlights safety and good tolerability of NXC139. **References:** [1] Boehm & Stahl, 2007. J. Nutr. Vol 137. [2] Oliveira *et al.*, 2015.
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Ex-vivo screening assay of pre- and probiotic combinations for the inhibition of pathogenic *Escherichia coli* in sows

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Introduction. Although pro-/prebiotics have been widely used in pig production, there are still inconsistent results on their efficacy [1, 2]. Beneficial effects depend not only on the probiotic strains or the prebiotic structure, but also on dose, animal type, health status, sanitary conditions, diet and age [3, 4]. Therefore, pro-/prebiotics should be applied in a targeted way, which includes conditions on individual farms. The aim of this study was to develop an ex-vivo screening assay to find tailor-made combinations of pro- and prebiotics for individual farms for the inhibition of enteropathogenic *Escherichia coli (EPEC)*.

Animals, material and methods. Fecal samples were obtained from 20 German pig farms. Three samples from each farm were used for ex-vivo analyses. Three probiotics (*Bacillus licheniformis* and *Bacillus subtilis*, *Saccharomyces cerevisiae boulardii*, *Enterococcus faecium*) and three prebiotics (Inulin, Fructooligosaccharide, Mannan-oligosaccharide) were selected alone or in combination to test their effectiveness to inhibit the growth of an EPEC model strain ("Abbotstown") in fecal samples. Thus, a total of 16 ex-vivo assays was performed for each sample. Fecal sample slurries were mixed with pro-/prebiotics and the *EPEC* strain and incubated anaerobically for 24h at 37°C. Aliquots of the incubated slurries were transferred to a medium containing a specific antibiotic mixture. During incubation for 24 h at 37°C, growth was recorded (OD690nm) in a microplate reader. Comparison of exponential growth phase OD values from pre-/ probiotic supplementation to controls without supplementation were used to analyze the survival of the EPEC strain. A mixture of three antibiotics (cloxacilline, metronidazole and vancomycin) was used to ensure selective growth of the EPEC model strain in the fecal slurries. The specificity was confirmed by the absence of growth in all fecal samples not inoculated with the model strain.

Results and discussion. Analysis of the growth curves showed that the time point of 8 h was most suitable to compare *EPEC* growth in controls to pre-/probiotic supplemented samples. Suppression of *E. coli* growth was not always observed, as some pro-/prebiotic combinations also led to increased growth of the model strain in some samples. Our results indicate that the survival of the model strain depended not only on the added pre/probiotics, but also on the origin of the fecal samples used. Thus, in-farm variation of *E. coli* growth was much lower than differences between farms.

Conclusion. The ex-vivo assay depends on a suitable antibiotic mixture to allow specific growth of a pathogen model strain after incubation with pre-/probiotics. The use of fecal samples includes the response of the individual microbiota and is therefore preferred to in -vitro assays. The developed method has the potential to find tailor-made combinations of pro - and prebiotics for individual farms to inhibit pathogenic *E. coli*, as many combinations can be tested in parallel.

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Nutritional management of dogs with heart disease: the owner's behavior

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Introduction. Dietary orientation is essential to properly manage dogs with heart disease (HD) but it is not always possible to owners to follow the correct nutritional guidance to the patient [1]. This study evaluated the owner's behavior on the nutritional management of dogs with HD.

Animals, material and methods. 96 owners of dogs with HD assessed in São Paulo-Brazil were interviewed. Body condition score (BCS) [2] and muscle mass score (MMS) [3] were evaluated. Data was analyzed by descriptive statistics.

Results and discussion. Dogs had 11.77±0.3 years of mean age and were 54% males and 46% females of different breeds. The most frequent diagnosis was chronic degenerative mitral valve disease (92.70%; specifically in stage C, followed respectively by stages B2, B1 and D). Other cardiopathies were arrhythmias, patent ductus arteriosus, dilated cardiomyopathy, pulmonary stenosis and cor triatriatum dexter. Regarding the BCS, 23.95% had low BCS (range: 1 - 3) while 21.87% were overweight (BCS 6 - 9). Muscle loss was observed in most dogs (56.25% moderate, 21.88% mild and 4.17% severe). Half of them (54.16%) maintained body weight over the last year, and 76.04% had normorexia. Meanwhile, weight loss was observed in 29.16% and anorexia or hyporexia in 20.83% of dogs. Fat and muscle mass loss are expected in patients with chronic diseases because of the rise in circulating inflammatory cytokines, which increase energy expenditure, protein degradation, and loss of appetite [4]. After diagnosis, only 7.27% of dogs consumed a prescription diet. Food intake was controlled in only 29.16% of dogs and from those, 64.32% followed a recommendation (veterinarian / feed label). Adequate energy consumption is important to maintain BCS and to promote higher survival rate [5] and owners should be instructed to attend to it specifically in a disease condition. Treats were provided to 77.08% of dogs. Medication was provided with human food in 62.5% of cases. Homemade food or wet food were provided as a flavoring for 33.34% of dogs. Most owners (78.12%) believed that sodium could negatively impact health but only 11.45% verified its content in feed provided. Despite the concern, owners still provide high sodium foods to their dogs, without considering it in daily total Na intake. Na intake should be restricted to 0.08 to 0.25% of dry matter in dogs with HD [6].

Conclusion. A significant portion of dogs had malnutrition (weight loss or overweight). The owners are concerned with the sodium intake levels but do not observe its inclusion in the label and provide excess of snacks, often with high amounts of sodium.

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Interaction effect of phase feeding, space allowance and mixing on productive performance of grow-finisher pigs

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Introduction. Space allowance (SA), mixing (M) and phase feeding (PF) are common management strategies in pig production. A low SA or M pigs can result in lower productive performance (PP) and affect nutrient requirements. This study aimed to quantify the effect of SA, M and PF on PP of grow-finisher pigs.

Animals, materials and methods. Three trials (T) were carried out. In T1 and T2, 345 pigs were moved as litters to the finisher stage at 11wks of age (32.5±4.1 kg) and were assigned to two different SA: 0.96 m2/pig (n=15 pens; 10 pigs/pen) and 0.78 m 2/pig (n=15 pens; 13 pigs/pen). Mixing was applied to 5 pens of each SA leading to a 2×2 factorial arrangement with SA and M as treatments. At 15wks of age (58.4±5.8 kg), PF was applied to 5 pens of each SA (not mixed) leading to another 2×2 factorial arrangement with two treatments: SA and diet (0.80 or 0.92% SID Lys). In T3, 230 pigs were moved to the finisher stage at 11wks of age (32.9±5.9 kg) and assigned in the same two SA with M in 20 pens. PF was applied at 15wks of age (53.7±9.0 kg) to 5 pens of each SA leading to a 2×2 factorial arrangement with SA and diet (0.80 or 0.92% SID Lys) as treatments. BW was recorded per pen every 2wks until 21wks of age. Feed intake was recorded daily per pen. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated and analysed using GLM with pen as the experimental unit. The model included initial BW, diet or M, SA and their interaction as fixed effects. Alpha for determination of significance and trends were 0.05 and 0.10 respectively.

Results and discussion. In T1 and T2, 0.80% SID Lys diet pigs (LD) had lower final BW (100.5±1.09 kg), lower ADG (1.07±0.03 kg) and higher FCR (2.46±0.05) than 0.92% SID Lys diet pigs (HD; 103.9±1.17 kg; 1.16±0.03 kg; 2.31±0.05) by the end of the trial (P<0.05). Non-M pigs were heavier (105.5±1.80 kg) and had higher ADG (1.01±0.03 kg) and ADFI (2.20±0.05 kg) than M pigs (99.8±1.82 kg; 0.93±0.03 kg; 2.08±0.05 kg) at the end of the trial (P<0.05). In T3, PP was not affected by diet and SA on the overall period of the trial (P>0.05). Nevertheless, BW, ADG and ADFI tended to show an interaction between diet and SA after 2wks of changing the diet (P<0.10). LD with low SA had lower BW (67.1±0.36 kg) and ADG (0.89±0.02 kg) than HD with high SA (68.7±0.36 kg; 1.00±0.02 kg) or low SA (69.6±0.36 kg; 1.06±0.02 kg; P<0.05). LD with high SA had lower BW (67.7±0.36 kg) and ADG (0.93±0.02 kg) than HD with low SA (69.6±0.36 kg; 1.06±0.02 kg; P<0.05). LD with low SA had lower ADFI (2.04±0.02 kg) than HD with low SA (2.24±0.02 kg; P<0.05).

Conclusion. Mixing and dropping SID Lys from 0.92 to 0.80% at 60 kg BW, have an impact on PP. Performance may be affected due to an interaction between PF and SA after 2wks of changing the diet in mixed pigs. Further work is needed to fully understand whether PF, SA and M interact each other.

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